LEVEL OF MATERNAL ANTIBODIES AGAINST RESPIRATORY SYNCYTIAL VIRUS (RSV) NUCLEOPROTEIN AT BIRTH AND RISK OF RSV VERY-SEVERE LOWER RESPIRATORY TRACT INFECTION

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Abstract

Background: The nucleoprotein (N protein) of respiratory syncytial virus (RSV) is a candidate antigen for new RSV vaccine development. The aim of the present study was to investigate the association between maternal antibody titers against the RSV N protein at birth and the newborns' risk of developing very-severe lower respiratory tract infection (VS-LRTI). Methods: In this single-center prospective cohort study, 578 infants born during the RSV epidemic season in France were included. Among these, 36 were hospitalized for RSV VS-LRTI. A generalized linear model was used to test the occurrence of a VS-LRTI in function of sex, mode of delivery, parity of the mother, type of pregnancy, date of birth in relation to the peak of the epidemic, and antibody titer against N protein. Results: All cord blood samples had detectable antibodies against N protein. The mean titers were significantly lower in newborns with risk factors for RSV severe LRTI (preterm infants, birth before the peak epidemic, multiparous mother). There was no association between antibody titer against the N protein and a protection against VS-LRTI. Conclusions The present study found that transfer of maternal antibodies against the RSV N protein may not provide a significant immune protection early in infancy. Clinical Trials Registration. NCT04144816.

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Methods: In this single-center prospective cohort study, 578 infants born during the RSV epidemic season in France were included. Among these, 36 were hospitalized for RSV VS-LRTI. A generalized linear model was used to test the occurrence of a VS-LRTI in function of sex, mode of delivery, parity of the mother, type of pregnancy, date of birth in relation to the peak of the epidemic, and antibody titer against N protein.

Results: All cord blood samples had detectable antibodies against N protein. The mean titers were significantly lower in newborns with risk factors for RSV severe LRTI (preterm infants, birth before the peak

epidemic, multiparous mother). There was no association between antibody titer against the N protein and a protection against VS-LRTI.

Conclusions The present study found that transfer of maternal antibodies against the RSV N protein may not provide a significant immune protection early in infancy.

Clinical Trials Registration . NCT04144816.

Introduction

Respiratory syncytial virus (RSV) is the leading cause of bronchiolitis in infants and of pneumonia in children younger than 5 years of age in infants [1,2], and in this population it is responsible each year for approximately 33 million cases of lower respiratory tract infections (LRTI) worldwide [1]. It is recognized to cause substantial mortality in low and middle-income countries [2], and burden in high-income countries [3,4]; the highest rate of hospitalization is in infants aged less than 3 months [5]. Despite this, preventive measures are to date limited to the monthly injection of RSV-specific neutralizing antibodies for children at a high risk of severe complications, the cost of which may be prohibitive [6]. There are, however, vaccines currently in development that have the potential to be a more affordable option to reduce the worldwide RSV burden [6]. Most candidate vaccines target the fusion protein, which is an envelope protein highly conserved between different subtypes or RSV, stabilized in its prefusion form [7,8]. However, RSV nucleoprotein (N protein), which it is implicated in nucleocapsid-RNA complex formation that allows RSV replication and transcription [9], is one of the most conserved viral protein among strains [10] and is also considered as a potential target for candidate vaccines inducing T CD8+ response [11,12]. These vaccines may be good candidates for maternal immunization strategy; an approach that appears as safe because of the particular vulnerability of infants and their inability to produce effective antibodies [13]. Such a strategy has already demonstrated its ability to provide a passive humoral protection for infants against tetanus, influenza, and pertussis [14]. Humoral protection is conferred at birth by the transfer of maternal IgG, the titer of which depends on the maternal level of RSV antibodies and the effectiveness of transplacental RSV antibody transfer from mother to infant [15]. Although the protection conferred to infants by neutralizing antibodies induced by natural exposure to RSV of mothers has been suggested, published data is conflicting [16–20]; furthermore there is no published data concerning the protection provided by maternal antibodies against the N protein. The aim of the present study was therefore to investigate the association between maternal antibody titers against the RSV N protein at birth and the newborns' risk of developing RSV VS-LRTI early in infancy.

Methods

Study design

All infants born at the Hôpital Femme Mère Enfant (HFME; part of the university hospitals of Lyon, France) from August 26, 2019 to February 27, 2020 (during the RSV epidemic season in France [4]) with a cord blood sample available were eligible for inclusion in this single-center prospective observational cohort study. Cord blood samples were collected for all included patients. Stillborn children, those living outside the Auvergne-Rhône-Alpes region or those with insufficient sample were excluded.

The administrative registry of all infants born in the university hospitals of Lyon (Hospices Civils de Lyon, HCL) was used to recover the following variables of interest: sex (male/female), month of birth, gestational age (weeks of amenorrhea, WA), maternal parity (primiparity/multiparity), type of pregnancy (simple/multiple gestation), mode of delivery (vaginal birth/caesarean section), and birth weight. Preterms were classified as either "moderate-to-late preterm" [from 32 to 36(+6) WA], "very preterm" [28 to 31(+6) WA], and "extremely preterm" [22 to 27(+6) WA], as defined by the World Health Organization (WHO) [21]. Low birth weight was defined as less than 2500 g [22].

Infants aged 3 months or younger who visited the emergency department of the HFME hospital (either directly or transfer from another hospital) between August 26, 2019 to May 27, 2020 with laboratory-confirmed RSV bronchiolitis recognized by the International Statistical Classification of Diseases and Related Health Problems (ICD-10) codes (J21.0, J12.1 and/or J20.5) were identified in the informatics' database of the

hospital. A 3-month follow-up seemed to be optimal to detect a protection purposed by maternal antibodies as children under 3 months of life experience the greatest risk of hospitalization and mortality [5,23], and as previous studies have shown that maternal antibody concentrations against RSV decreased between 2 and 6 months [1,24], with a half-life of approximately 27 days [25]. According to local protocols, all infants hospitalized with an LRTI diagnosis were tested for RSV on a nasopharyngeal sample. Laboratory-confirmed RSV infection were those diagnosed by real-time reverse transcriptase (RT)-PCR, as previously described [26]. Clinical records were reviewed to further classy these as VS-LRTI, as defined by the WHO: cough or difficulty breathing, associated with fast breathing or peripheral capillary oxygen saturation (SpO2) $< 90\%$ or inability to feed or unconscious [13]. The peak of the epidemic was defined as December 9, 2019 based on both pediatric emergency admissions for bronchiolitis and RSV detection data in Lyon, in accordance with data from previous years.

Laboratory procedures

Cord bloods were collected at birth in heparinized tubes, centrifuged and stored at -20°C until testing. IgG against RSV N protein were measured in 1:100 diluted plasma using an in-house enzyme-linked immunosorbent assay (ELISA) performed according to Roux *et al.*[27] and using recombinant N protein from RSV A long strain and anti-human polyvalent Ig G/A/M peroxidase antibody produced in goat (Sigma-Aldrich®, Saint-Louis, MA, US). In order to establish a standard curve, 8 two-fold dilutions (starting at 1:100) of a positive control (BEI Resources NR-4020, Manassas, VA, US) were used. Negative control was obtained from the IgM/IgG RSV ELISA kit (VIROTECH Diagnostics, Dietzenbach, HE, DE). Titers were calculated using a Michaelis-Menten equation applied to the values of the standard curve and expressed as relative unit (RU)/mL.

Statistical analysis

Mean and standard deviation (SD) were calculated to describe clinical continuous variables. Concerning antibody titers against RSV N, both mean (SD) and median (interquartile range, IQR) were calculated. Comparisons between mean antibody titers were tested using a 2-sided parametric Student t-test or an ANOVA for more than 2 groups. The birth weight and the gestational age were treated as categorical variables. A generalized linear model (GLM) was used to test the occurrence of a VS-LRTI in function of sex, mode of delivery, parity of the mother, type of pregnancy, date of birth in relation to the peak of the epidemic, and titer antibodies against RSV N (quantitative variable). Preterm infants as well as patients who contracted a VS-LRTI but for whom the result for RT-PCR RSV detection was negative were excluded from the GLM. Statistical analyses were performed using R software version 4.0.3.

Ethics

This study was approved by the review board of university hospitals of Lyon, is registered on ClinicalTrials.gov (NCT04144816), and was declared to national data protection commission (19-198).

Results

Characteristics of the cohort

During the study period 1719 infants were born at the HFME. Cord bloods were available for 1574 of them. Because it was not financially feasible to test all samples, two days, every two months, were allocated to process study samples, and then 578 were randomly included for analysis. Among those included, there were 71 infants aged 0-3 months who visited the emergency department with a diagnosis of LRTI and 43 hospitalized with a diagnosis of VS-LRTI; of the latter, 36 tested positive for RSV (Fig. 1).

The total cohort comprised 578 infants; 53.8% (n=311) were boys, 6.1% (n=35) from multiple births, 23.0% (n=133) born by cesarean section, from 60.7% (n=351) multiparous mothers, 9.9% (n=57) born preterm, and the mean (SD) birth weight was 3179 (633) g. Among the 36 hospitalized in their first 3 months of life with a laboratory-confirmed RSV VS-LRTI infection, 50.0% (n=18) were boys, none were premature, 88.9% (n=32) were born to multiparous mothers, and none were from multiple births (Table 1).

Maternal antibody titers against the RSV N protein

The mean (SD) value of maternal antibody titers against the RSV N protein among newborns was 502 (255) RU/mL. There was no significant difference in mean values according to sex (Figure 2A), type of pregnancy (Figure 2B), or mode of delivery (Figure 2C). The mean value was significantly different according to the term of pregnancy ($p < 0.001$; Figure 2D); it was significantly lower in preterm newborns (i.e. extremely, very, and moderate-to-late combined) than in those born at term (377 RU/mL vs. 516 RU/mL, $p < 0.001$), in newborns with a low birth weight than in those with a birth weight [?] 2500g (401 RU/mL vs. 515 RU/mL, $p < 0.001$; Figure 2E), in newborns from primiparous mother than those from multiparous mothers (470 RU/mL vs. 522 RU/mL, $p < 0.05$; Figure 2F) and in newborns whose birth occurred before the peak of the epidemic than in those born after (458 RU/mL vs. 542 RU/mL, $p < 0.001$; Figure 2G). There was no significant difference in mean antibody titers between infants who experienced a VS-LTRI (irrespective of RSV detection) and those who did not (466 RU/mL vs. 505 RU/mL, $p = 0.37$; Figure 2H).

Prediction of VS-LRTI using clinical variables and serological status

Preterm infants were excluded from the GLM given that a 3-month follow-up was not sufficient to analyze their risk of being admitted to hospital for VS-LRTI (they might have been hospitalized since birth at 3 months of age). In multivariate analysis, maternal multiparity (Relative Risk, RR: 2.34, 95%CI [1.58; 3.01]) and a date of birth before the peak of the epidemic (RR: 2.84, 95%CI [2.08; 3.59]) were significantly associated with the occurrence of VS-LRTI; there was no significant association between antibody titer and VS-LRTI (Table 2).

Discussion

In the present study no association was found between antibody titer against the RSV N protein measured in cord blood and a protection against RSV VS-LRTI. This result differs from the largest cohort studies that analyzed cord blood titers; these studies, conducted in Denmark, Mali, and on American Indian infants found that a higher concentration of neutralizing antibodies at birth was associated with a lower risk of contracting an RSV LRTI and of being hospitalized for this [14,15,23]. Considering the power of the present study; the sample size is comparable with the study reported by Buchwald *et al.*(587 infants) [18], and greater than that reported by Eick *et al.* (372 infants) [16], which both concluded to a protection conferred by neutralizing antibodies (while the study conducted in Alaska [155 infants] and Kenya [90 infants] did not find any association [17,18]). Therefore, only a weak protection might have been missed. In addition, the statistical power was sufficient to detect a robust association between known risk factors of RSV VS-LRTI (parity of the mother and a date of birth in the first part of the epidemic season [29]) and the occurrence of VS-LRTI. For the present study, owing to the lack of data available regarding antibody titers against RSV N protein, there was no determination of number of subjects required; the data provided herein will therefore help for the power calculation in future studies.

It is of note that the mean antibody titers against RSV N protein at birth were lower in premature infants, those with a low birth weight, born to a primiparous mother, and born before the peak of the epidemic. This profile is not surprising; for instance, it is well known that majority of transplacental transfer of IgG occurs during the third trimester of pregnancy, and that preterm newborns have a compromised passive immunity [28–30]. Furthermore, multiparous mothers are likely to have been exposed to RSV, as well as those delivering at the end of the epidemic season. These factors are also known to be risk factors for severe RSV infection [31–33]. The multivariate analysis indicates that this risk is mostly mediated through another pathway that the lack of RSV N antibodies.

The study does have some limitations. The most important is that only patients admitted to the hospital they were born in were considered for the analysis of VS-LRTI, thus any cases visiting elsewhere may have been missed. However, there are few other pediatric emergency departments in the region, the HFME university hospital is the largest pediatric hospital in the area and the only one with an intensive care unit; thus it is likely that most cases of RSV VS-LRTI occurring before 3 months of age were captured from either direct admission or from transfers from other hospitals. Furthermore, detection used ELISA that allows

standardization of result but does not quantify neutralizing activity. However, a neutralization assay would not have specifically detect antibodies against N protein which were investigated herein. Finally, the severity of LRTI episodes might have been overestimated; a third of cases (n=12/36) were classified as VS-LRTI only on the "inability to feed" criterion, and patients hospitalized for LRTI were very likely to have enteral nutrition, sometimes for less than twenty-four hours (*data not shown*). However it is unlikely to have led to a differential selection according to the antibody titer level, and the classification of all episodes of LRTI according to the WHO standardized severity criteria allows comparison with future studies.

In conclusion, the findings presented herein suggest that transfer of maternal antibodies against the RSV N protein does not provide a significant immune protection early in infancy against RSV VS-LRTI. Maternal vaccination may therefore not be a suitable strategy for an N protein candidate vaccine.

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References:

1. Shi T, McAllister DA, O'Brien KL, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. Lancet. **2017** ; 390(10098):946–958.

2. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet. **2010** ; 375(9725):1545–1555.

3. Zhou H, Thompson WW, Viboud CG, et al. Hospitalizations associated with influenza and respiratory syncytial virus in the United States, 1993-2008. Clin Infect Dis. **2012** ; 54(10):1427–1436.

4. Kramer R, Duclos A, VRS study group in Lyon, Lina B, Casalegno J-S. Cost and burden of RSV related hospitalisation from 2012 to 2017 in the first year of life in Lyon, France. Vaccine. **2018** ; 36(45):6591–6593.

5. Stockman LJ, Curns AT, Anderson LJ, Fischer-Langley G. Respiratory syncytial virus-associated hospitalizations among infants and young children in the United States, 1997-2006. Pediatr Infect Dis J.**2012** ; 31(1):5–9.

6. Simoes EAF, Bont L, Manzoni P, et al. Past, Present and Future Approaches to the Prevention and Treatment of Respiratory Syncytial Virus Infection in Children. Infect Dis Ther. **2018** ; 7(1):87–120.

7. Karron RA. Preventing respiratory syncytial virus (RSV) disease in children. Science. **2021** ; 372(6543):686–687.

8. Graham BS. Vaccine development for respiratory syncytial virus. Curr Opin Virol. **2017** ; 23:107–112.

9. Bakker SE, Duquerroy S, Galloux M, et al. The respiratory syncytial virus nucleoprotein–RNA complex forms a left-handed helical nucleocapsid. J Gen Virol. **2013** ; 94(Pt 8):1734–1738.

10. Collins PL, Fearns R, Graham BS. Respiratory syncytial virus: virology, reverse genetics, and pathogenesis of disease. Curr Top Microbiol Immunol. **2013** ; 372:3–38.

11. Abarca K, Rey-Jurado E, Munoz-Durango N, et al. Safety and immunogenicity evaluation of recombinant BCG vaccine against respiratory syncytial virus in a randomized, double-blind, placebo-controlled phase I clinical trial. EClinicalMedicine. **2020** ; 27:100517.

12. Green CA, Scarselli E, Voysey M, et al. Safety and immunogenicity of novel respiratory syncytial virus (RSV) vaccines based on the RSV viral proteins F, N and M2-1 encoded by simian adenovirus (PanAd3-RSV) and MVA (MVA-RSV); protocol for an open-label, dose-escalation, single-centre, phase 1 clinical trial in healthy adults. BMJ Open. **2015** ; 5(10):e008748.

13. Modjarrad K, Giersing B, Kaslow DC, Smith PG, Moorthy VS, WHO RSV Vaccine Consultation Expert Group. WHO consultation on Respiratory Syncytial Virus Vaccine Development Report from a World Health Organization Meeting held on 23-24 March 2015. Vaccine. **2016** ; 34(2):190–197.

14. Jones CE, Calvert A, Le Doare K. Vaccination in Pregnancy-Recent Developments. Pediatr Infect Dis J. **2018** ; 37(2):191–193.

15. Wilcox CR, Holder B, Jones CE. Factors Affecting the FcRn-Mediated Transplacental Transfer of Antibodies and Implications for Vaccination in Pregnancy. Frontiers in Immunology. **2017** ; 8:1294.

16. Eick A, Karron R, Shaw J, et al. The role of neutralizing antibodies in protection of American Indian infants against respiratory syncytial virus disease. Pediatr Infect Dis J. **2008** ; 27(3):207–212.

17. Stensballe LG, Ravn H, Kristensen K, et al. Respiratory syncytial virus neutralizing antibodies in cord blood, respiratory syncytial virus hospitalization, and recurrent wheeze. J Allergy Clin Immunol.**2009** ; 123(2):398–403.

18. Buchwald AG, Graham BS, Traore A, et al. RSV neutralizing antibodies at birth predict protection from RSV illness in infants in the first three months of life. Clin Infect Dis. **2020** ; ciaa648.

19. Bulkow LR, Singleton RJ, Karron RA, Harrison LH, Alaska RSV Study Group. Risk factors for severe respiratory syncytial virus infection among Alaska native children. Pediatrics. **2002** ; 109(2):210–216.

20. Nyiro JU, Sande CJ, Mutunga M, et al. Absence of Association between Cord Specific Antibody Levels and Severe Respiratory Syncytial Virus (RSV) Disease in Early Infants: A Case Control Study from Coastal Kenya. PLoS One. **2016** ; 11(11):e0166706.

21. Blencowe H, Cousens S, Oestergaard MZ, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. **2012** ; 379(9832):2162–2172.

22. Kramer MS, Papageorghiou A, Culhane J, et al. Challenges in defining and classifying the preterm birth syndrome. Am J Obstet Gynecol.**2012** ; 206(2):108–112.

23. Giersing BK, Karron RA, Vekemans J, Kaslow DC, Moorthy VS. Meeting report: WHO consultation on Respiratory Syncytial Virus (RSV) vaccine development, Geneva, 25-26 April 2016. Vaccine. **2019** ; 37(50):7355–7362.

24. Capella C, Chaiwatpongsakorn S, Gorrell E, et al. Prefusion F, Postfusion F, G Antibodies, and Disease Severity in Infants and Young Children With Acute Respiratory Syncytial Virus Infection. J Infect Dis.**2017** ; 216(11):1398–1406.

25. Buchwald AG, Graham BS, Traore A, et al. RSV neutralizing antibodies at birth predict protection from RSV illness in infants in the first three months of life. Clin Infect Dis. **2020** ; ciaa248.

26. Gaymard A, Bouscambert-Duchamp M, Pichon M, et al. Genetic characterization of respiratory syncytial virus highlights a new BA genotype and emergence of the ON1 genotype in Lyon, France, between 2010 and 2014. J Clin Virol. **2018** ; 102:12–18.

27. Roux X, Dubuquoy C, Durand G, et al. Sub-nucleocapsid nanoparticles: a nasal vaccine against respiratory syncytial virus. PLoS One.**2008** ; 3(3):e1766.

28. Linder N, Ohel G. In utero vaccination. Clin Perinatol.**1994** ; 21(3):663–674.

29. Mammas IN, Drysdale SB, Rath B, et al. Update on current views and advances on RSV infection (Review). Int J Mol Med. **2020** ; 46(2):509–520.

30. Malek A, Sager R, Kuhn P, Nicolaides KH, Schneider H. Evolution of maternofetal transport of immunoglobulins during human pregnancy. Am J Reprod Immunol. **1996** ; 36(5):248–255.

31. Homaira N, Mallitt K-A, Oei J-L, et al. Risk factors associated with RSV hospitalisation in the first 2 years of life, among different subgroups of children in NSW: a whole-of-population-based cohort study. BMJ Open. **2016** ; 6(6):e011398.

32. Bont L, Checchia PA, Fauroux B, et al. Defining the Epidemiology and Burden of Severe Respiratory Syncytial Virus Infection Among Infants and Children in Western Countries. Infect Dis Ther. **2016** ; 5(3):271–298.

33. Aikphaibul P, Theerawit T, Sophonphan J, Wacharachaisurapol N, Jitrungruengnij N, Puthanakit T. Risk factors of severe hospitalized respiratory syncytial virus infection in tertiary care center in Thailand. Influenza Other Respir Viruses. **2021** ; 15(1):64–71.

Table 1: Clinical characteristics of the population.

	Total cohort (N = 578)	Cases of VS-LRTI (N = 36)
Sex		
Female	267 (46.2%)	18 (50.0%)
Mode of delivery		
Vaginal	445 (77.0%)	29 (80.6%)
Caesarean	133 (23.0%)	7 (19.4%)
Parity		
Monoparity	227 (39.3%)	4 (11.1%)
Multiparity	351 (60.7%)	32 (88.9%)
Gestational age at delivery (weeks of amenorrhea)		
Mean (SD+)	38.7 (2.5)	39.1 (1.2)
Term	521 (90.1%)	36 (100.0%)
Moderate-to-late preterm	43 (7.4%)	0 (0.0%)
Very preterm	9 (1.6%)	0 (0.0%)
Extremely preterm	5 (0.9%)	0 (0.0%)
Infant birth weight (g)		
Mean (SD+)	3179 (633)	3301 (337)
Low birth weight (< 2500 g)	65 (11.3%)	0 (0.0%)
Type of pregnancy		
Singleton	543 (93.9%)	36 (100.0%)
Multiple	35 (6.1%)	0 (0.0%)
Date of birth in relation to the peak of the epidemic		
Birth before the peak	277 (47.9%)	31 (86.1%)
Birth after the peak	301 (52.1%)	5 (13.9%)

+SD = Standard Deviation

Table 2. Multivariate analysis of the factors associated with VS-LRTI

	Level	Relative risk (RR) of VS-LRTI+	[95% CI]
Sex	Female	Ref	
	Male	0.89	[0.34; 1.4
Type of pregnancy	Singleton	Ref	
	Multiple	0.14	[-1.42; 1.4
Mode of delivery	Vaginal	Ref	
	Caesarean	0.88	[0.14; 1.6

	Level	Relative risk (RR) of VS-LRTI+	[95% CI]+
Parity	Monoparity	Ref	
	Multiparity	2.34	[1.58; 3.0
Date of birth in relation to the peak of the epidemic	Birth after the peak	Ref	
	Birth before the peak	2.84	[2.08; 3.5
Titer of antibodies	Slope	-0.00049	[-0.00167

**p< 0.01, ***p< 0.001. Relative risk and p values are calculated using a generalized linear model

+VS-LRTI = very severe low respiratory tract infection.

++95% CI = 95% confidence interval

Figure 1: Flow chart. LRTI: Lower Respiratory Tract Infection.

Figure 2: Titers of antibodies against N protein in different subgroups A. Sex, B. type of pregnancy, C.Mode of delivery, D. Gestational age at delivery, E. Infant birth weight, F. Parity, G. Date of birth (epidemic peak), H. VS-LTRI. Boxplots present medians and interquartile ranges [IQR], wiskers 1.5 times the IQR, and outliers are $>$ 1.5 times and $<$ 3 times the interquartile range beyond end of the box. *p $<$ 0.05, **p $<$ 0.01, ***p $<$ 0.001 in Student t-test or ANOVA.