Specific immunoglobulin A levels against RSV and its clinical characteristics in a cohort of infected pediatric patients in Mexico

Angel Sanchez Tinajero[1], Ma Isabel Salazar Sánchez[2], Manuel Castillejos-Lopez[1], Danna Patricia Ruiz Santillán [1], José Arturo Martínez Orozco[1], Eduardo Becerril Vargas[1], Joél Velázquez Pérez[1], and Elizabeth González Cueto[2]

[1]Instituto Nacional de Enfermedades Respiratorias
[2]Escuela Nacional de Ciencias Biologicas

July 1, 2020

Abstract

Introduction: Respiratory Syncytial Virus (RSV) is the main causative agent of respiratory tract infections at pediatric ages in Mexico and around the world. In our study we determine and correlating clinics characteristics to the levels of specific IgA against RSV in respiratory samples for infected children and the absence or presence of exclusive breastfeeding. Material and methods: This is a clinical, ambispective and comparative study. There were included all patients pediatrics hospitalized and the samples of nasopharyngeal exudate were obtained, there was performed an ELISA on them with a Human Respiratory Syncytial Virus to determine the levels of specific IgA against RSV. Results: The total of patients included was 197. Finding that only 4.1% of infants received a complete vaccination schedule. Up to 22.8% were coinfected with a virus upon admission. There was found a significant difference in the rate of oxygenation during the admission presented by lactated vs non-lactated patients (p 0.002), as well as the presence of crackles p = 0.029 (95% CI 0.502-0.095) and wheezing p = 0.043 (95% CI 0.522-0.980), and a difference between the levels of specific IgA and the personal history of repeated respiratory tract infections (p = 0.018). Differences in symptoms such as dry cough, thoracoabdominal dissociation (p = 0.043, p = 0.044 respectively), were found as well. Conclusions: The results obtained from this research lead to stablish that low levels of specific IgA against RSV in the airways, are statistically and clinically related to greater severity of RSV infection.

Introduction

Respiratory Syncytial Virus is an Orthopneumovirus, member of the family*pneumoviridae.* It is a single-stranded nonsegmented negative-sense RNA virus, class V of the Baltimore classification. There are distinguished two RSV subtypes, to know: A and B, each of which has 5 to 6 subgroups, with subtype A being the most prevalent.[8]

RSV is the main causative agent of respiratory tract infections in pediatric ages in Mexico and around the world.[8] It is considered the main cause of hospitalizations in this age group, eliciting 3.4 million hospitalizations anually in children under 5 years old worldwide. [17] The morbidity and mortality figures for this pathology are similar, or even higher than those caused by the Influenza virus, reaching an annual mortality rate estimated at 3.1 cases per 100,000 people younger than 1 year. [17,19]

RSV is responsible for almost 100% of episodes of bronchiolitis and up to 50% of viral infections of the pulmonary parenchyma in pediatric age. [8]

Up to 10% of infected children with this virus can present severe forms of the disease such as pneumonia or pulmonary focus sepsis.[19]

Bronchiolitis is the characteristic clinical-pathological entity of RSV infection. It is defined as the first episode of wheezing in infants younger than 1 year [29], accompanied by mild symptoms such as nasal congestion, hyaline rhinorrhea, cough, tachypnea, rales, fever, irritability, or even data on respiratory failure or sepsis, all this tributary to a state of inflammation, hypersecretion of mucus, edema and necrosis of respiratory epithelial cells precipitated by the cellular infection produced by this virus. [22]

Risk factors that predispose to severe RSV infection can be categorized into: maternal, environmental, and host factors. The main factors identified are, but not limited to: prematurity (due to a decrease in the number of antibodies transplacentally acquired, transmitted from the mother to the newborn), low birth weight (<2500gr according to the Mexican Clinical Practice Guidelines), genetic abnormalities, cardiopulmonary comorbidity and immunocompromises.[4,29]

The diagnosis is primarily clinical, however, in situations of seriousness it is necessary to determine the specific etiology of the condition, since RSV infection is clinically indistinguishable from other viral respiratory infections and the diagnosis requires laboratory tests, so it is necessary to carry out complementary studies such as real-time PCR. [4]

So far, therapeutics are limited to support measures such as oxygen therapy, Ribavirin (an ineffective synthetic nucleoside with serious teratogenic effects) [3], and a humanized monoclonal antibody called Palivuzumab, which in addition to its low availability in Mexico and its high cost, is only indicated in patients with risk factors such as prematurity and with underlying comorbidities, not for those with outpatient treatment. [1]

Lumicitabine also known as ALS-8176 is a new prodrug, nucleoside analogue currently in phase II of development, which selectively inhibits the activity of RNA polymerase through the termination of the classical chain. [8] This drug significantly reduced the level of contagion and the detection time of viral titers in blood without provoking any side effects.

The lack of a safe and effective treatment to cope with RSV infection requires the implementation of prophylactic measures, of which breastfeeding is considered the main one. [4]

The role of breastfeeding in the contribution of humoral immunity in the newborn has been extensively described, through the passage of immunoglobulins (especially IgA type) through breast milk, likewise, it has recently been exposed that in addition to transmitting humoral-type immunity, cellular immunity such as anti-inflammatory cytokines and TGF-β, which regulates the differentiation and expansion of innate and adaptive immune cells, are also transmitted. [5]

IgA represents 90% of the immunoglobulins in the breast milk (BM), with a concentration of 0.3g / ml / day. It is present in mucosa, has anti-inflammatory components, and even inactivate viruses. IgM is the second most important immunoglobulin, it is highly avid for viruses and bacteria due to its pentameric nature, having an opsonizing function. Its concentration in BM is 2.5mg / ml, it can be concentrated in mucosa when IgA levels decrease, other immunologically relevant components in breast milk are enzymes and complement, as well as lymphocytes and cytokines.

RSV infections are a serious health challenge in Mexico and in the world, which until now does not have a safe, effective and standardized treatment. Some new treatments (Lumicitabine and vaccine prototypes) seem promising as future therapeutic schemes, however, they are still in development stages II.

In our study, we determined the clinical characteristics of hospitalized RSV-infected pediatric patients, relating these characteristics to the absence or presence of exclusive breastfeeding in the first 6 postnatal months, as well as the levels of virus-specific IgA in respiratory samples.

Material and methods

This is an ambispective, observational, descriptive, and comparative clinical research study to determine the association between levels of specific immunoglobulin A against respiratory syncytial virus (RSV) and clinical characteristics in pediatric patients infected with this virus. There were included all hospitalized pediatric

patients younger than 5 years old with a diagnosis of RSV pneumonia by Luminex multiple molecular assay and RT-PCR according to the CDC Atlanta protocol, identified within the Clinical Microbiology laboratory from the period of 2014 to 2017, as well as respiratory samples (nasopharyngeal exudate / bronchioalveolar lavage) from infected children in the period from August 2018 to August 2019 at the National Institute of Respiratory Diseases (INER). There were considered non-inclusion criteria all patients who were diagnosed with RSV infection in another laboratory, who were not found in the age limit, as well as all those patients for whom it was not achieved to complete the samples or information necessary to meet the objectives of this study.

Statistic analysis

The frequencies and proportions of the qualitative and median variables and their respective measure of dispersion (interquartile ranges) of quantitative variables were analyzed, according to their type of distribution determined by the Kolmogorov-Smirnov or Shapiro-Wilk test, depending on the case. The collection of the data was carried out in pairs.

In the bivariate analysis, the variables were compared using χ^2 or Fisher's exact test when nature was qualitative, and the Mann-Whitney test when they were quantitative variables (average levels of immunoglobulin A), and CI were also determined by Odds ratio to determine risk factors, the p values were also calculated in the design, which allowed us to know a possible association between the levels, being considered significant when the p value was [?]0.05.

There was generated a database in which the information collected from each patient was included and the univariate, bivariate and stratified analysis were performed, using the SPSS version 20.0 software.

Ethical considerations

Since the data was obtained from secondary sources (clinical records), and from the respiratory samples directly from the clinical laboratory, no procedure or intervention was performed with the patients, so the risk was considered minimal. Informed consent was not required and patient confidentiality will be safeguarded.

The present study was carried out in accordance with the Helsinki declaration and international good practices, preserving the complete confidentiality of all the information, it will be protected and will be available only to researchers using identification codes.

Results

There were analyzed 450 files of pediatric patients diagnosed with RSV infection existing in the INER database, from May 2014 to June 2017, of which 167 were within the age limit set in the study.

In the prospective period, a total of 70 infected patients were identified, from August 2018 to September 2019, of whom 41 were within the delimited age limit in this study. Only 30 samples of nasopharyngeal exudate were available.

The total sum of patients included in this study was 197, of which clinical, laboratory, radiographic, and outcome variables (mechanical ventilation requirements, mortality, and hospitalization days) were analyzed. There were determined the levels of specific IgA against RSV from the 30 samples of nasopharyngeal exudate, by direct ELISA test, which was performed in triplicate (Table 5), a procedure already detailed in the methodology, obtaining the following results:

A male predominance of 59.9%. The patients who received exclusive breastfeeding for 6 months were 90, which corresponds to 45.7%. (Table 1)

Only 4.1% of infants received a complete vaccination schedule corroborated with a vaccination card.

The main symptoms found in this population were: rhinorrhea runny nose, fever, cough and irritability in order of frequency, 72.8%, 71.6%, 59.9% and 59.3% respectively. Solely 30 patients (15.2%) presented some

kind of gastrointestinal symptom, such as nausea and vomiting, one of whom had febrile seizures. (Table 1)

Regarding the personal pathological history of the patients included in this research, it was found that up to 46.7% presented clinical symptoms of previous wheezing or bronchiolitis, as well as repeated respiratory tract infections (more than 3 episodes per year) in 34%. In 42.6% corresponding to 84 patients, it was identified that at least one of the 2 parents had a regular smoking habit, and it was also found that up to 41.6% of pediatric patients showed a previous medical history with a diagnosis of gastroesophageal reflux disease and a 12.2% of asthma.

Twenty patients, which corresponds to 10.2% of the total, were premature at birth (<34 weeks gestation). Only 3 patients presented some risk factor for immunosuppression and 11 (5.6%) reported bronchopulmonary dysplasia or some cardiac malformation, with patent ductus arteriosus and Ebstein's anomaly being the reported ones.

Regarding respiratory distress data, during the physical examination, it was found that up to 75.1% of the patients presented intercostal retractions and 50.3% thoracoabdominal dissociation. The oxygenation index at admission was determined as moderate in a 43.1%. The severity of bronchiolitis was classified as moderate to severe in 19.8% of cases.

As outcomes, it was obtained that up to 22.8% were coinfected with some virus upon admission, being the Rhinovirus the main causative agent of said coinfection, up to 10.7%, followed by Seasonal influenza and Parainfluenza1 virus in 4.6% of cases. . In 1 patient, the 2009 H1N1 pandemic influenza virus was isolated and considered as clothing. (Table 1).

Likewise, it was identified that 23.4% of the children were coinfected with some bacterium, of which, in 11.7% it was Pneumococcus, followed by H. infuenzae by 5.1%. and C. pneumoniae in 1%.

About bacterial nosocomial coinfections, 2% of the patients presented them, being E. coli, Pseudomonas A. and S. aureus the isolated organisms, in 1%, 0.5% and 0.5% of cases respectively. Fungal coinfections were also found in 1 of the patients, in which Aspergillus spp was isolated as the responsible fungus.

The median age, weight and height were 12 months, 10Kg, and 77cm respectively. Physical examination revealed a median O2 saturation of 86%. The median hospitalization days was 7 days, 18 patients were transferred to the intensive care unit requiring invasive mechanical ventilation, which lasted up to 10 days in 1 patient. (Table 2)

Regarding laboratorials, the medians for the hematic biometry were as follows: 12 gr / dl for in hemoglobin, 37% hematocrit and 306 thousand platelets per microliter. Regarding the white series, a median of 8.7 thousand per microliter of leukocytes was found, with neutrophils being predominant in 57% on average, followed by lymphocytes in 31%. The mean of the creatinine levels was 0.3 mg / dl, likewise the CPK levels were 84 mg / dl and the lactic dehydrogenase 288 Ul / L as average.

The C-reactive protein was found in concentrations of 0.76 mg / L as average and the measured levels of procalcitonin were 0.15 ng / ml as average.

Comparative statistics

In the statistical comparison, there was found a significant difference in the oxygenation index at admission presented by lactated vs. non-lactated patients (p 0.002), as well as the presence of crackles p = 0.029 (95% CI 0.502-0.095) and wheezing p = 0.043 (95% CI 0.522-0.980). (Table 3)

The comparative analysis of the medians of these averages was performed, finding a statistically significant difference between the levels of specific IgA and the personal history of repetitive respiratory tract infections (defined according to the Mexican CPG as more than 3 respiratory infections per year) (p = 0.018). Differences were also found in symptoms such as: dry cough (figure 2), presence of severity signs such as thoracoabdominal dissociation (figure 3), (p = 0.043, p = 0.044 respectively). (Table 4)

DISCUSSION

The data obtained in this investigation indicate that there is a direct statistically and clinically significant relationship between the levels of specific immunoglobulin A against Respiratory Syncytial Virus in respiratory samples (nasopharyngeal exudate) and the level of severity of the clinical picture presented by pediatric patients infected with this agent, the presence of serious clinical variables such as thoraco-abdominal dissociation and dry cough which are more common in those whose antibody levels are lower. The afore mentioned agrees with what was published by Walsh et al (2018), who measured IgG antibodies against RSV F protein in infected patients, finding in a multivariate analysis adjusted for age, a significant correlation between the decrease in clinical severity and the increase in titers of the antibodies, with greater benefit, in the first 2 months of age, also finding that the greater antibodies titers at birth are correlated with delayed RSV infection, thus suggesting the benefits of maternal vaccination during pregnancy. [28]

Likewise, in 2017, Jans et al, also correlated the levels of antibodies against the RSV G protein and the presence of severity markers such as oxygen therapy need, tachypnea, oxygen saturation, admission to the intensive care unit and days of hospitalization, finding that the avidity of RSV-specific IgG antibodies was lower in vitro in infected infants than in uninfected controls, though, they found no relationship between the titers of these antibodies and the severity data.[10] However, Falsey, Koval, DeVincenzo and Walsh (2017), demonstrated that high titers of neutralizing antibodies against RSV bring benefits in the survival of immunocompromised patients infected with this virus [7] also Nyiroet al. (2016), reported the association between high titers of neutralizing antibodies against RSV, measured in the umbilical cord of pediatric patients with severe infection and the decrease in the probability of acquiring the infection over time in controls. [18] However, they mention a wide variation in antibody levels, which is why further studies will follow in this regard. [10,7, 18]

These investigations support that, the greater the number of specific antibodies against the respiratory syncytial virus, (both IgG and IgA), the clinical data on the severity of said infection in pediatric populations will be lower.

Previous research regarding RSV antibody levels are all measured in serum, Vissers, Ahout, de Jonge and Ferwerda (2016), supported that elevated levels of IgG in infected infants mucosa are correlated with a lower viral load than plasma IgG levels, suggesting focusing humoral immunity studies on mucosae of patients affected by this virus, corroborating in this study what was stated by these authors.[27]

Likewise, there was also found a relationship between the presence of clinical signs of repetitive respiratory tract infections in pediatric patients whose RSV-specific immunoglobulin A titers were lower.

Jounai et al. (2017), examined the responses of total IgG antibodies, subclasses and antibodies against the neutralizing epitopes of patients in acute and convalescent stages of RSV infection, also evaluated the serum neutralizing activity, finding that children antibody responses against RSV matured over time in terms of titer, neutralization levels, increased affinity, and increased overall IgG response in repeat virus infections (Jounai et al., 2017). ([12])

On the other hand, another of the results obtained in this research indicates that there is a relationship between breastfeeding for 6 months and the decrease in the presence of clinical severity data in RSV infection, such as low oxygenation index, presence of crackles and wheezes on physical examination. This is recently supported by what was published in January of this year in Nepal by scientists who carried out a clinical study where the concentration of immunoglobulins A and G against RSV F fusion protein was measured in breast milk, finding that patients with active RSV infection had lower IgG concentrations than controls, ,being the comparison of means statistically significant. This supports the important role played by the antibodies present in breast milk as a protective measure against infections and symptoms of this virus. [17]

In regard to the clinical characteristics epidemiology of Mexican pediatric patients, data that are not updated in Mexico, it was found that there was a predominance of males, the median age was 12 months, this in contrast to what was reported by Rodríguez-Auadetal., in 2012, where it is mentioned that the median age in their study population was 22 months. Most of the patients presented incomplete vaccination schedule, which, at present, represents a real public health problem, probably due to the social stereotypes focused

on vaccination, a situation which, focused on the infection context by RSV, predisposes to situations of co-infections, increased mortality and risk of RSV infection, making visible the lack of optimization of vaccination tools and strategies. [11,22]

Also, it was documented in this investigation that the patients presented as important pathological personal history, exposure to tobacco smoke, repeated respiratory tract infections, previous wheezing, gastroesophageal reflux disease and asthma. 18 patients who had some underlying disease were treated in the intensive care unit and mechanically intervened for breathing, data that is consistent with that published by Vissers, Ahout, de Jonge, and Ferwerda (2016), who also mention the predominance of these conditions in patients younger than 6 months of age. [27]

This study showed that the main coinfections identified among the included patients were essentially viral, with Rhinovirus being the main responsible agent, followed by seasonal influenza and Parainfluenza 1 virus, this coinciding with what was reported by Robledo-Acevedo et at ., last year. [22] A high exposure to cigarette smoke was also found in the population of patients included in this research, as was also found by these authors. About the coinfections found, Pneumococcus was the most frequently isolated bacterium in this population, as well as the H1N1 influenza virus, whose association RSV-Influenza H1N1 was already reported by Taylor et al. (2017).[26] Finally Aspergillus spp. it was the only isolated fungus considered as a fungal coinfection in this study.

The clinical relevance of the findings in this research lies in the importance of promoting breastfeeding as a preventive method to avoid severe RSV infections in pediatric patients, since currently there is no effective vaccine or treatment. and standardized to prevent and treat such infection. Likewise, the medical importance of the information produced by this research updates the epidemiological data of the population affected by this infection and its clinical characteristics and outcomes.

The limitations of this research are related to the methodological procedure for measuring specific immunoglobulin A against RSV, since when trying to measure these levels in a poorly studied medium (nasopharyngeal exudate), it is a priority to standardize this process and to indicate by means of ROC curves, the cut points of said concentrations, opening a chain of subsequent investigations that can determine specific concentrations of specific IgA and even propose it as a diagnostic or monitoring method in infected patients.

Conclusions

The results of this research confirm that patients with low specific IgA levels present more frequently: general malaise, cough and thoracoabdominal dissociation. Low levels of RSV-specific IgA in the airways are statistically and clinically related to increased severity of RSV infection. Clinical variables such as the low oxygenation index, the presence of crackles and wheezing, of pediatric patients with RSV infection are more common in those who did not breastfeed for 6 months. Breastfeeding is a protective factor to avoid developing a severe picture of RSV pneumonia and with it, the potential death, so it should be considered as a powerful preventive weapon.

References

American Academy of Pediatrics Committee on Infectious Diseases. (2014) *Updated Guidance for Palivizumab Prophylaxis Among Infants and Young Children at Increased Risk of Hospitalization for Respiratory Syncytial Virus Infection.* [Versión electrónica].Pediatrics, 134(2):e620-38. Doi: 10.1542/peds.2014-1666.

Brady, M., Byington, C., Davies, H., Edwards, K., Jackson, M., Maldonado, Y.(2014). *Updated Guidance for Palivizumab Prophylaxis Among Infants and Young Children at Increased Risk of Hospitalization for Respiratory Syncytial Virus Infection.* [Versión electrónica]. Pediatrics, 134(2), e620-38. Doi: 10.1542/peds.2014-1666.

Carande, E., Pollard, A., Drysdale, S. (2016*). Management of respiratory syncytial virus bronchiolitis: 2015 survey of members of the European Society for Paediatric Infectious Diseases* . [Versión electrónica]. The

Canadian journal of infectious diseases & medical microbiology = Journal canadien des maladies infectieuses et de la microbiologie medicale, 2016, 919139537. Doi: 10.1155/2016/9139537

Diagnóstico y tratamiento de bronquiolitis aguda en niñas/niños y en el primer nivel de atención. Guía de evidencias y recomendaciones: Guía de práctica clínica. México, Cenetec; 2015 (consultada el 31 de octubre de 2019). Disponible en: http://www.cenetec.salud.gob.mx/interior/catalogoMaestroGPC.html

Pamela P., Carneiro M., (2017). *Immunology of breast milk.*[Versión electrónica]. Rev Assoc Med Bras (1992). 2016 Sep;62(6):584-593. doi: 10.1590/1806-9282.62.06.584.

Hashimoto K, Hosoya M. (2017). *Neutralizing epitopes of RSV and palivizumab resistance in Japan* . [Versión electrónica]. Fukushima J Med Sci. 2017 Dec 19;63(3):127-134. doi: 10.5387/fms.2017-09. Epub 2017 Sep.

Falsey, A., Koval, C., DeVincenzo J., Walsh, E. *(2017). Compassionate use experience with high-titer respiratory syncytical virus (RSV) immunoglobulin in RSV-infected immunocompromised persons* . [Versión electrónica].Transplant infectious disease: an official journal of the Transplantation Society, 19(2), 1-8. Doi: 10.1111/tid.12657.

Gamiño-Arroyo, A., Moreno-Espinosa S., Llamosas-Gallardo B, Ortiz-Hernández A, Guerrero M, Galindo-Fraga A., et al. (2017).*Epidemiology and clinical characteristics of respiratory syncytial virus infections among children and adults in Mexico* . [Versión electrónica]. Influenza and other respiratory viruses, 11(1), 48-56. Doi: 10.1111/irv.12414.

Hui, D., Rossi, G., Johnston, S. (2016). *SARS, MERS and other Viral Lung Infections.* European Respiratory Society publications. 72, 84-109. Doi: 10.1183/2312508X.erm7216

Jans, J., Wicht, O., Widjaja, L., Ahout, L., de Groot, R., Guichelaar, T., et al. *(2017). Characteristics of RSV-Specific Maternal Antibodies in Plasma of Hospitalized, Acute RSV Patients under Three Months of Age.* [Versión electrónica].PLoS One,12(1):e0170877.Doi.org/10.1371/journal.pone.0170877

Javelle, E., Colson, P., Parola, P., Raoult, D. (2019). *Measles, the need for a paradigm shift* . [Versión electrónica]. European journal of epidemiology, 1573-7284. Doi: 10.1007/s10654-019-00569-4.

Jounai, N., Yoshioka, M., Tozuka, M., Inoue, K., Oka, T., Miyaji, K., et al. (2017*). Age-Specific Profiles of Antibody Responses against Respiratory Syncytial Virus Infection* . [Versión electrónica].EBioMedicine, *16* , 124–135. Doi:10.1016/j.ebiom.2017.01.014.

Lee, N., Qureshi, S. (2013). Other Viral Pneumonias Coronavirus, Respiratory Syncytial Virus, Adenovirus, Hantavirus. [Versión electrónica]. Critical care clinics, 29(4), 1045-1068. Doi: 10.1016/j.ccc.2013.07.003

Liu, W., Chen, D., Tan, W., Xu, D., Qiu, S., Zeng, Z., et al. (2016). Epidemiology and Clinical Presentations of Respiratory Syncytial Virus Subgroups A and B Detected with Multiplex Real Time PCR. [Versión electrónica]. PLoS One, 11(10), e0165108. Doi: 10.1371/journal.pone.0165108.

Lukšić, I., Kearns, P., K., Scott, F., Rudan, I., Campbell, H., Nair, H. (2013). *Viral etiology of hospitalized acute lower respiratory infections in children under 5 years of age – a systematic review and meta-analysis.* . [Versión electrónica]. Croatian medical journal*, 54(2), 122–134. Doi:10.3325/cmj.2013.54.122*

Mazur, N., Horsley, N., Englund, J., Nederend, M., Magaret, Kumar A., et al. (2019). *Breast Milk Prefusion F Immunoglobulin G as a Correlate of Protection Against Respiratory Syncytial Virus Acute Respiratory Illness* . [Versión electrónica]. The journal of infectious diseases, 219(1), 59-67. Doi: 10.1093/infdis/jiy477.

Noor, A., Krilov, L. (2018). *Respiratory syncytial virus vaccine: where are we now and what comes next?* [Versión electrónica]. Expert opinion on biological therapy,18(12), 1247-1256. Doi: 10.1080/14712598.2018.1544239

Nyiro, J., Sande, C., Mutunga, M., Kiyuka, P., Munywoki, P., Scott, J., et al. (2016). *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. [Versión electrónica].PLoS One, 11(11), e0166706. Doi: 10.1371/journal.pone.0166706.

Patel, K., Kirkpatrick C., Nieforth, K., Chanda, S., Zhang, Q., McClure, M., et al. (2019). *Respiratory syncytial virus-A dynamics and the effects of lumicitabine, a nucleoside viral replication inhibitor, in experimentally infected humans.* [Versión electrónica]. The journal of antimicrobial chemotherapy, 74(2), 442-452. Doi: 10.1093/jac/dky415.

Palmeira, P., Carneiro-Sampaio, M. (2016). *Immunology of breast milk* . [Versión electrónica]. Revista da Associação Médica Brasileira, 62(6), 584-593. Doi: 10.1590/1806-9282.62.06.584.

Piedimonte, G. (2015). *RSV infections: State of the art.*[Versión electrónica]. Cleveland Clinic journal of medicine, 82 (11 Suppl 1). Doi: 10.3949/ccjm.82.s1.03

Robledo-Aceves, M., Moreno-Peregrina, M., Velarde-Rivera, F., Ascencio-Esparza, E., Preciado-Figueroa, F., Caniza, M., et al. (2018).*Risk factors for severe bronchiolitis caused by respiratory virus infections among Mexican children in an emergency department.*[Versión electrónica]Medicine, 97(9), e0057. Doi: 10.1097/MD.0000000000010057.

Sakaguchi, K., Koyanagi. A., Kamachi, F., Harauma, A., Chiba, A., Hisata, K., et al. (2018). *Βρεαστφεεδινγ ρεγυλατες δεελοπμεντ οφ ιμμυνε σψστεμ τηρουγη ΤΓΦ-β ιν μιςε πυπς.* [Versión electrónica]. Pediatrics international: official journal of the Japan Pediatric Society, 60(3):224-231. Doi: 10.1111/ped.13507.

Simões, E., Bont, L., Manzoni, P., Fauroux, B., Paes, B., Figueras-Aloy, J., et. al. (2018). *Past, Present and Future Approaches to the Prevention and Treatment of Respiratory Syncytial Virus Infection in Children* . [Versión electrónica]. Infectious diseases and therapy, 7(1), 87–120. Doi: 10.1007/s40121-018-0188-z.

Shay, A., Holman, R., Newman, R., Liu, L., Stout, J., Anderson, L. (1999). *Bronchiolitis-associated hospitalizations among US children, 1980-1996.* [Versión electrónica]. The Journal of the American Medical Association, 282(15), 1440-6. DOI: 10.1001/jama.282.15.1440.

Shi T., David A McAllister, D., O'Brien, K., Simoes, E., Madhi, S., Gessner, B. (2017). *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.* [Versión electrónica]. Lancet, 390 (10098), 946–958.

Vissers, M., Ahout, I.,de Jonge, M., Ferwerda, G. (2016). *Mucosal IgG Levels Correlate Better with Respiratory Syncytial Virus Load and Inflammation than Plasma IgG Levels.* [Versión electrónica].Clinical and vaccine immunology, 23(3), 243-245. Doi: 10.1128/CVI.00590-15.

Walsh, E., Wang, L., Falsey, A., Qiu, X., Corbett, A., Holden-Wiltse, J., et al. (2018). *Virus-Specific Antibody, Viral Load, and Disease Severity in Respiratory Syncytial Virus Infection.* [Versión electrónica].The journal of infectious diseases, 218(2), 208-217. Doi: 10.1093/infdis/jiy106.

Xing, Y., Proesmans, M. (2019).*New therapies for acute RSV infections: where are we?* [Versión electrónica].European journal of pediatrics, 178(2),131-138.

Basal Characteristics	**Frecuency (n=197)**	**%**
Epidemiology	**Epidemiology**	**Epidemiology**
Female	79	40.1
Male	118	59.9
Breastfeeding (6 months)	90	45.7
Complete vaccination schedule (n=31)	8	4.1
Pathological personal history	**Pathological personal history**	**Pathological personal history**
Previous wheezing	92	46.7
Smoking parents	84	42.6
Gastroesophageal Reflux Disease	82	41.6

Previous bronchiolitis pictures	66	36.5
Prematurity (<34 weeks of pregnancy)	20	10.2
Low birth weight (<1500g)	15	7.6
Repeated airway infection (>3 per year)	67	34.0
Asthma	24	12.2
Obesity	9	4.6
Low weight	6	3.0
Immunosupression	3	1.5
Chromosomal syndromes	1	0.5
Steroid use	2	1.0
Renal disease	1	0.5
Signs and symptoms	**Signs and symptoms**	**Signs and symptoms**
Hyaline rhinorrhea	142	72.8
Fever	141	71.6
Productive cough	99	59.3
Oral intolerance	74	37.3
Dyspnea	27	13.7
Intercostal retractions	148	75.1
Pharyngeal hyperemia	138	70.1
Wheezing	108	54.8
Crackles	106	53.8
Thoracoabdominal dissociation	99	50.3
Nose flaring	87	44.2
Cyanosis	38	19.3
Oxygenation index at admission	**Oxygenation index at admission**	**Oxygenation index at admission**
Mild	76	38.6
Moderate	85	43.1
Severe	5	2.5
Bronchiolitis severity	**Bronchiolitis severity**	**Bronchiolitis severity**
Mild	149	75.6
Moderate	39	19.8
Severe	9	4.6
Clinical Presentation	**Clinical Presentation**	**Clinical Presentation**
Pneumonia	84	42.6
Pneumonia and isolation	46	23.4
Ventilator-associated pneumonia	3	1.5
Hospital-Acquired Pneumonia	2	0.5
Coinfections	**Coinfections**	**Coinfections**
Viruses	45	22.8
Rinovirus	21	10.7
Influenza H3N2	9	4.6
Parainfluenza	9	4.6
Influenza H1N1 PD09	1	0.5
Bacteria	46	23.4
S. pneumoniae	23	11.7
H. Influenzae	10	5.1
Bacterial after 48 hours of admission	4	2
E. coli	2	1
P. aeruginosa	1	0.5
S. aureus	1	0.5
Fungi	1	0.5

Aspergillus spp.	1	0.5

Table 1. Basal characteristics of the population.

Basal Characteristics 2 n=197	**Q25**	**Q50 (Median)**
Anthropometry and physical examination	**Anthropometry and physical examination**	**Anthropometry and**
Age (months)	0.6	12
Weight (Kg)	8	10
Height (cm)	68	77
O2 Saturation (%)	84	86
Laboratorials	**Laboratorials**	**Laboratorials**
Hemoglobin	11.3	12
Platelets	235	3.6
Leukocytes	6.3	8.7
Neutrophils	40.5	57
Lymphocytes	20	31
Creatinine	0.2	0.3
Creatine phosphokinase	60.2	84
Lactic dehydrogenase	246.2	288
C-Reactive Protein	0.21	0.76
Procalcitonin	0.1	0.15

Table 2. Basal characteristics of the population 2.

Variable	Breastfeeding YES (n=90)	Breastfeeding NO (n=76)	p (OR CI 95%)
Clinical picture	Clinical picture	Clinical picture	Clinical picture
Oxygenation index at admission	Mild 31 Moderate 55 Severe 4	Mild 45 Moderate 30 Severe	0.002 (-)
Crackles	59	31	0.029 (0.502-0.955)
Wheezing	53	32	0.043 (0.522-0.980)

Table 3. Comparison of breastfeeding for 6 months and clinical characteristics of infected pediatricians.

Variable		**Specific IgA concentration against *RSV* (UE/ml) n=30 Median**
Recurrent respiratory tract infection (> 3 per year)	Yes	0.67
	No	1.09
General discomfort	Yes	0.92
	No	3.07
Dry cough	Yes	0.42
	No	1.17
Thoraco-abdominal dissociation	Yes	0.79
	No	2.26

Table 4. Comparison of specific IgA levels against *RSV* and clinical characteristics of infected pediatrics.

Patient	Average concentration (EU/ml) of viral specific IgA	Patient	Average concentration (EU/ml) of viral specific I
1	4.33	**11**	0.67
2	2.00	**12**	0.11
3	3.07	**13**	0.29
4	2.25	14	0.42
5	0.62	15	1.75
6	0.97	16	0.30
7	0.75	17	3.87
8	0.54	18	0.67
9	1.17	19	0.37
10	3.36	20	0.26

Table 5. Average concentration (EU / ml) of specific IgA against RSV.