Low Interferon-γ and Interleukin-13 Levels in the Respiratory Tract are Related to Life-threatening Respiratory Syncytial Virus Infection in Previously Healthy Infants.

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Background: The mechanisms of interindividual susceptibility to respiratory syncytial virus (RSV) are not clearly understood. Viral load (VL), an exaggerated immune response or T-helper 2 (Th2) bias were postulated as playing a role in the severity of RSV infection. We aimed to analyze VL, cytokine profiles and their association with life-threatening disease (LTD).
Methods: Prospective cohort study including previously healthy full-term infants < 12 months old, hospitalized with a first RSV infection in 2017-2019. Nasopharyngeal aspirates were performed on admission to assess VL (by qRT-PCR) and cytokines levels (using a Bioplex panel). Cytokines were grouped according to their functional classification: proinflammatory (TNF-α), regulatory (interleukin [IL]-10), Th1 (interfereron-γ [IFN-γ]), Th2 (IL-4, IL-5, IL-9, IL-13) and Th17 (IL-17a). Patients were defined to have LTD when required admittance to the intensive care units and ventilatory support.
Results: One hundred and nineteen patients were studied, 68 (57%) were male; median age was 3 months. Nineteen (16%) infants developed LTD, with no significant differences in socioeconomic, pregnancy and infant variables compared with other RSV cases. Virus subtypes were not related to LTD (p= 0.77). VL was not associated with LTD (p= 0.51). Patients with LTD had significantly lower levels of IFN-γ (mean 0.08 pg/ml [standard deviation -SD- 0.06] vs. 0.32 pg/ml [SD 0.4]; p= 0.001) and IL-13 (mean 0.03 pg/ml [SD 0.04] vs. 0.05 pg/ml [SD 0.07]; p= 0.02), fig 1. IFN-γ and IL-13 inversely correlated with days of hypoxemia (p< 0.001 and p= 0.002, respectively). VL, IFN-γ and IL-13 did not correlate with duration of symptoms before admission. No relationship was observed between IFN-γ, IL-13 and age, sex or breastfeeding. In 102 (86%) samples, IL-4 was under the limit of quantitation. TNF-α, IL-5, IL-9, IL-10 e IL-17a were not related to LTD.
Conclusion:
- To our knowledge, this is the first study to relate low IL-13 levels to LTD in previously healthy infants.
- Patients with LTD had 4-fold lower levels of IFN-γ.
- The severity of RSV infection may be the result of a deficient immune response rather than a higher viral load or an overactive immune response.
- These findings provide additional evidence for the development of preventive and therapeutic strategies.

BACKGROUND

- Respiratory syncytial virus (RSV) is the most frequent agent of severe acute respiratory infection and one of the leading causes of mortality in infants worldwide.
- Most of RSV hospitalized infants are previously healthy with no known risk factors.
- The mechanisms of interindividual susceptibility to respiratory syncytial virus (RSV) are not clearly understood.
- Viral load (VL), an exaggerated immune response or T-helper 2 (Th2) bias were postulated as playing a role in the severity of RSV infection.

OBJECTIVE

- We aimed to analyze VL, cytokine profiles and their association with life-threatening disease (LTD) in previously healthy infants with RSV infection.

METHODS

- Prospective cohort study during 2017, 2018 and 2019 RSV seasons (May-September).
- Site: Hospital de Niños "Dr. Ricardo Gutiérrez", a tertiary pediatric hospital, reference center in Buenos Aires, Argentina, with ~9,500 annual admissions and ~450,000 annual outpatient visits.
- Inclusion criteria: previously healthy full-term infants under 12 months old, hospitalized with a first RSV infection.
- Nasopharyngeal aspirates were obtained on admission. RSV diagnosis and viral titers were assessed by RT-qPCR (Tib Molbiol LightMix® Kit, Germany). Cytokine levels were measured by multiplex immunoassay (Bioplex®, Bio-Rad Laboratories, USA).
- Cytokines were grouped according to their functional classification: proinflammatory (TNF-α), regulatory (interleukin [IL]-10), Th1 (interfereron-γ [IFN-γ]), Th2 (IL-4, IL-5, IL-9, IL-13) and Th17 (IL-17a).
- Patients were defined to have LTD when required admittance to the intensive care units and ventilatory support.

RESULTS

- One hundred and nineteen patients were studied, 68 (57%) were male; median age was 3 months. Characteristics of the study population are detailed in *Table 1.*

Table 1. Characteristics of the population.

Variable	LTD (n=19)	No LTD (n=100)	*P value*
Gestational age, in weeks, m_e (IQR)	39 (39-40)	39 (38-40)	0,074
Birth weight, in kilograms, m_e (IQR)	3,5 (3,1-3,7)	3,3 (3,1-3,6)	0,974
Age, in months, m_e (IQR)	3 (2-5)	3 (2-6)	0,518
Male, n (%)	9 (47)	59 (59)	0,350
Days from symptoms onset, m_e (RIC)	3 (2-4)	4 (3-5)	0,117
Breastfeeding, n (%)	14 (74)	83 (83)	0,342
Crowding, n (%)	10 (53)	46 (46)	0,622
Complete vaccination, n (%)	13 (68)	63 (63)	0,771
Maternal smoking, n (%)	1 (5)	12 (12)	0,428
Maternal asthma, n (%)	1 (5)	4 (6)	0,918

- Nineteen (16%) infants developed LTD, with no significant differences in socioeconomic, pregnancy and infant variables compared with other RSV cases.
- No differences were observed in the VL of patients with LTD compared with those patients with better outcome (p=0.51), *figure 1.* Viral load did not correlate with number of days of symptoms before collection of nasopharyngeal aspirates or the duration of supplemental oxygen requirement, *figure 2.*
- Virus subtypes were not related to LTD (p= 0.77). We found a positive correlation between VL and levels of IFN-γ (p=0,009), IL-5 (p=0,01), IL-10 (p=0,0002) y TNF-α (p=0,005).

Figure 1. Viral load and life-threatening disease.

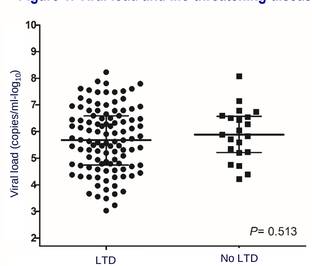
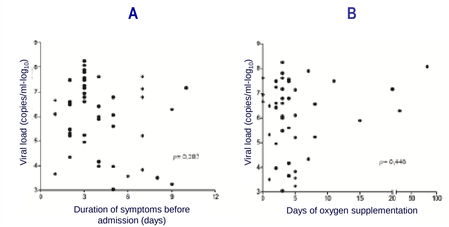


Figure 2. Analyses of viral load and its relationship with duration of symptoms (A) and supplemental oxygen requirement (B).



- Patients with LTD had significantly lower levels of IFN-γ (mean 0.08 pg/ml [standard deviation -SD- 0.06] vs. 0.32 pg/ml [SD 0.4]; p= 0.001) and IL-13 (mean 0.03 pg/ml [SD 0.04] vs. 0.05 pg/ml [SD 0.07]; p= 0.02), *figure 3.*
- IFN-γ and IL-13 inversely correlated with days of hypoxemia (p<0.001 and p=0.002, respectively), *fig. 4.*

Figure 3. Low IFN-γ and IL-13 levels were related to life-threatening disease.

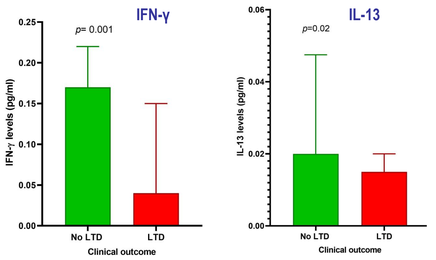
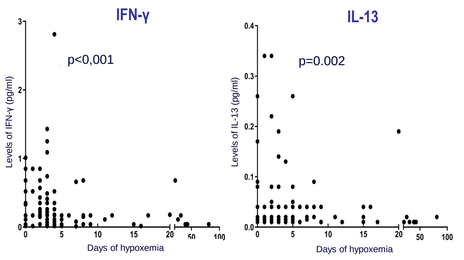
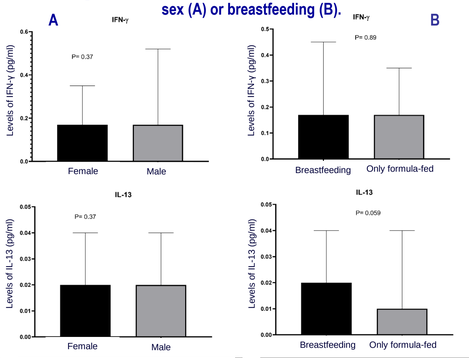


Figure 4. IFN-γ and IL-13 levels inversely correlated with days of hipoxemia.



- VL, IFN-γ and IL-13 did not correlate with duration of symptoms before admission (p=0,84; p=0,08).
- No correlation was observed between IFN-γ, IL-13 and age (p=0,12; p=0,29).
- We found no association between IFN-γ, IL-13 and sex or breastfeeding, *figure 5.*
- TNF-α, IL-5, IL-9, IL-10 e IL-17a were not related to LTD.
- In 102 (86%) samples, IL-4 was under the limit of quantitation.

Figure 5. IFN-γ and IL-13 levels were not associated with sex (A) or breastfeeding (B).



CONCLUSIONS

- To our knowledge, this is the first study to relate low IL-13 levels to LTD in previously healthy infants.
- Patients with LTD had 4-fold lower levels of IFN-γ.
- The severity of RSV infection may be the result of a deficient immune response rather than a higher viral load or an overactive immune response.
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