

Supplementary materials

Reference	Study design and population, Specimen collection, Exclusion criteria	Sample size and number of cases	Outcome – measure of interest	Results (p value)
[8]	Prospective study on hospitalized children (10 d – 4 y, median age 4 mo) with RSV ARI, in a subgroup of 19 infants < 11 mo NW at admission and every 1-3 d throughout hospitalization with RSV culture (\log_{10} TCID ₅₀ /mL), No exclusion criteria	59 patients, 19/59 infants with NW, 17/19 with pneumonia \pm bronchiolitis, 2/19 with bronchiolitis	Presence of pulmonary consolidation, Hypoxia, LOS	Significant correlation between greater quantities of RSV and pulmonary consolidation ($p < 0.025$), no significant correlation between greater quantities of RSV and hypoxia and/or prolonged LOS ($p > 0.05$)
[9]	Prospective study on hospitalized < 2 years children with RSV infection, nasal aspirate at admission with RSV culture (\log_{10} PFU/mL) Exclusion criteria: BPD, hemodynamically significant CHD, ID, SCS treatment within the 3 weeks before admission, Ribavirin treatment, RSV immunoglobulin or RSV-specific Ab treatment within the 3 months before enrollment, need for MV for apnea	39 patients, 15/39 severe bronchiolitis (= need for MV during hospitalization), 24/39 non-severe	Disease severity (= need for MV during hospitalization)	Significant correlation between higher nasal RSV quantities and severe bronchiolitis ($p = 0.022$)
[11]	Prospective observational study on previously healthy children < 2 years hospitalized for RSV bronchiolitis, nasal aspirate at admission with RSV culture (\log_{10} PFU/mL) Exclusion criteria: BPD, ID, hemodynamically significant CHD, SCS treatment in the preceding month, positive blood cultures	141 patients	LOS, Need for PICU, need for MV	Significant correlation between higher nasal RSV quantity and longer LOS ($p = 0.008$; a 1 log PFU/mL higher RSV load predicted 0.8 days longer LOS), need for PICU ($p = 0.0049$), need for MV ($p = 0.0048$)
[12]	Prospective study on previously healthy infants < 1 year hospitalized for RSV bronchiolitis, NPA (once for patient) with RSV load by RT-PCR (RSV-RNA transcript/ μ L) Exclusion criteria: BPD, Hemodynamically significant CHD, ID, SCS treatment within the 3 weeks before admission, Ribavirin treatment, RSV-specific Ab preparations within the 3 months before enrollment	81 patients, 28/81 with risk factors for severe disease (prematurity and/or LBW), 53/81 without risk factors	Severe disease intended as: - RR > 60 bpm, - LOS > 7 days, - Need for PICU, - Need for MV	Significant correlation between higher RSV load and severe bronchiolitis in both patients with risk factors ($p = 0.024$) and without risk factors ($p = 0.027$)
[13]	Prospective observational study on previously healthy infants < 6 mo outpatients with first RTI, NPA at enrollment with viral load expressed by PCR-CT values (inverse linear relationship between viral load and CT-values) Exclusion criteria: NA	82 infants, 30/82 infants with RSV infection, 11/30 infants with RSV as sole pathogen	Specified disease severity score	Significant correlation between RSV viral load and disease severity score value in infants with RSV infection ($p = 0.003$) even when RSV as sole pathogen ($p = 0.02$)
[14]	Prospective monocentric study on previously healthy children \leq 2 years	219 patients,	Hospital admission,	Significant correlation between higher RSV load

	with RSV infection, NPA or ET aspirate at admission and daily until discharge, RSV load (\log_{10} PFU/mL) Exclusion criteria: BPD, hemodynamically significant CHD, ID, SCS treatment within the preceding month, positive blood cultures	192/219 hospitalized 218/219 samples on day 1, 102/219 samples on day 2, 41/219 samples on day 3	LOS, Need for PICU, Need for MV	and prolonged LOS ($p < 0.001$; RSV load increase of 1 log PFU/mL predicted 1 additional day in LOS), between higher RSV load on day 3 and need for PICU ($p = 0.009$), between reduced clearance (Δ viral load d 1-3) and prolonged LOS ($p = 0.035$)
[16]	Prospective study on children < 3 years (median age 2.2 mo) hospitalized for RSV bronchiolitis, NPW at admission, RSV load (log copy number of RSV-RNA/mL) Exclusion criteria: Underlying chronic condition (prematurity, BPD, CF, CHD, ID) and recurrent (more than one) wheezing episodes	132 patients	Specified disease severity score, LOS	Significant correlation between higher RSV load and higher value of severity score ($p = 0.024$) and between higher RSV load and prolonged LOS ($p = 0.038$)
[17]	Prospective study on previously healthy children ≤ 2 years hospitalized for bronchiolitis, NPA at admission, day 3, day 5, day 7, RSV load (\log_{10} copy number of RSV-RNA/mL) Exclusion criteria: Recurrent wheezing, Ribavirin or SCS treatment in the preceding two weeks	60 patients, 40/60 with RSV bronchiolitis (age < 1 year), 18/40 severe bronchiolitis, 18/40 moderate bronchiolitis, 4/40 mild bronchiolitis	Specified disease severity score	Significant correlation between higher RSV load and higher disease severity score value ($p < 0.001$), significant differences in the mean RSV load between the mild group and the severe group ($p = 0.014$)
[18]	Prospective multicenter study on children < 2 years hospitalized for bronchiolitis, NPA at admission, RSV load expressed by PCR-CT values (inverse linear relationship between viral load and CT-values) Exclusion criteria: Enrollment in previous studies, Enrollment > 48 hours since hospital admission	2615 patients, 1764/2615 RSV infection, 587/1764 low RSV genomic load, 598/1764 medium load, 579/1764 high load, 1223 patients with a stricter definition of bronchiolitis (age ≤ 1 year, GA ≥ 37 weeks)	LOS, need for PICU	Significant correlation between higher RSV genomic load and risk of LOS ≥ 3 days (OR 1.58, $p < 0.001$), even in the subgroup with a stricter definition (OR 1.46, $p = 0.01$), significant correlation between higher RSV genomic load and PICU (OR 1.43, $p = 0.03$), without correlation in the subgroup without a stricter definition ($p > 0.05$)
[19]	Double-blinded RCT (inhaled racemic adrenaline vs. saline) on infant < 1 year hospitalized for bronchiolitis with specified severity score values ≥ 4 (moderate-severe illness), NPA at admission with RSV load expressed by PCR-CT values (inverse linear relationship between viral load and CT-values) Exclusion criteria: presence of any serious cardiac, immunologic, neurologic, oncologic, pulmonary disease other than bronchiolitis, >1 previous episode of obstructive airway disease, symptoms of disease of	404 patients, 363/404 patients with NPA, 300/363 RSV infection, 200/363 high RSV load, 89/363 RSV single infection	LOS, need for oxygen therapy, need for ventilatory support	Significant association between higher RSV load and prolonged LOS ($p = 0.042$), need for oxygen therapy ($p = 0.006$) or ventilatory support ($p = 0.021$)

	the lower airway (e.g. coughing) for >4 weeks, An CS treatment in the preceding 4 weeks			
[22]	Observational analytical cross-sectional study on infants < 1 year hospitalized for RSV LRTI, NPA < 3 d or ≥ 3 d from illness with RSV genomic load expressed by PCR-CT values (inverse linear relationship between viral load and CT-values) Exclusion criteria: Prematurity, ID, documented chronic lung or heart disease, possible bacterial infection	86 infants with RSV LRTI 49/86 moderate clinical score disease severity values, 37/86 severe clinical score disease severity values	Specified clinical score disease severity	Significant correlation between higher RSV genomic loads and severe RSV LRTI in later LRTI stage (NPA ≥ 3 d; p = 0.04)
[25]	Prospective study on infants 0-6 mo hospitalized for RSV±RV bronchiolitis, NPA at admission with RSV load expressed by PCR-CT values (inverse linear relationship between viral load and CT-values) Exclusion criteria: Recent (≤ 30 d before current infection) or current SCS treatment, Recent (≤ 15 d before current infection) or current antibiotic treatment, Previous wheezing crisis, CHD, BPD	70 patients, 61/70 infants RSV infection, 49/61 sole-RSV infection, 12/61 RSV+RV infection	LOS, ICU stay, Oxygen therapy duration, Respiratory support duration	In patients with sole-RSV infection, higher RSV load is a risk factor for prolonged LOS (OR 3.23, p = 0.012), prolonged ICU stay (OR 2.03, p = 0.036), prolonged oxygen therapy duration (OR 2.96, p = 0.011), prolonged respiratory support duration (OR 1.84, p = 0.021); in patients with RSV+RV infection, a higher RSV load is a risk factor for prolonged oxygen therapy duration (OR 4.78, p = 0.017)

Table S1. Studies with results supporting a direct relation between RSV load and bronchiolitis severity.

Note: Ab = antibodies, ARI = acute respiratory infection, BPD = bronchopulmonary dysplasia, bpm = breaths per minute, CF = cystic fibrosis, CHD = congenital heart disease, CPAP = Continuous Positive Airways Pressure, CT = cycle threshold, d = days, ED = Emergency Department, ET = endotracheal tube, GW = General Ward, ID = immunodeficiency, IVIG = intravenous immunoglobulin, LBW = low birth weight, LOS = length of stay, LRTI = low respiratory tract infection, mo = months, MV = mechanical ventilation, NA = not available, NPA = nasopharyngeal aspirate, NPW = nasopharyngeal wash, NW = nasal wash, PFU = plaque-forming units, PICU = Pediatric Intensive Care Unit, RTI = respiratory tract infection, RT-PCR = Real Time Polymerase Chain Reaction, SCS = systemic corticosteroids, RR = respiratory rate, RSV = Respiratory Syncytial Virus, RV = Rhinovirus, SatO₂ = oxygen saturation, TCID₅₀ = 50% tissue culture infectious dose, URTI = upper respiratory tract infection, w = weeks, y = years.

Reference	Study design and population, Specimen collection, Exclusion criteria	Sample size and number of cases	Outcome – measure of interest	Results (p value)
[23]	Prospective cross-sectional study on children ≤ 2 years with diagnosis of bronchiolitis in ED, NW at enrollment with copy numbers measurement of 5 different RSV genes (NS1, NS2, N, G and F) No exclusion criteria	112 patients, 79/112 with RSV infection, 41/79 hospitalized patients, 38/79 outpatients	Hospitalization, SatO ₂	Significant correlation between higher RSV gene copy numbers (except F) and non-hospitalization (p = 0.01 – 0.04); significant correlation between higher RSV gene NS1, NS2, N copy numbers and higher SatO ₂ (p = 0.03)
[24]	Prospective study on children 2 w – 2 y and weight > 2 Kg (also with non-respiratory comorbidities, e.g. prematurity) hospitalized with PCR-confirmed viral bronchiolitis, NPA up to twice daily during hospitalization with RSV load (RSV-RNA copies/mL) Exclusion criteria: any local or systemic factor that could influence the safety of nasal sampling (e.g., bleeding disorders), presence of nasal cannulae in both nostrils, palliative care, respiratory comorbidity	55 patients, 30/55 patients with RSV, 12/30 severe (PICU), 18/30 moderate (GW)	Severity disease defined by the type of hospitalization (PICU or GW)	Significantly higher RSV load in moderate than in severe cases (p = 0.001), with a significant decreasing in viral load in severe RSV cases relative to moderate ones (p = 0.001)
[7]	Prospective study on healthy children < 2 years hospitalized or evaluated in outpatient setting for RSV infection, nasal swab only at admission in outpatients, once a day during hospitalization, with RSV load (log ₁₀ copy number of RSV-RNA/mL) Exclusion criteria: duration of illness >7 days, prematurity, underlying medical conditions (i.e. CHD, BPD, ID), use of immunomodulatory drugs (including SCS) > 5 days within 2 weeks of presentation, previous history of wheezing or hospitalization for RSV bronchiolitis	150 children with RSV infection, 111/150 hospitalized 39/150 outpatients,	Specified clinical severity score, Hospitalization, Need for oxygen therapy	Significant correlation between higher RSV load and outpatients (p = 0.003), lower clinical score value in outpatients (p = 0.0002), lower need of oxygen therapy in hospitalized patients (p = 0.04)

Table S2. Studies finding an inverse correlation between RSV load and bronchiolitis severity.

Reference	Study design and population, Specimen collection, Exclusion criteria	Sample size and number of cases	Outcome – measure of interest	Results (p value)
[10]	Multicenter double-blind placebo-controlled RCT on children ≤ 2 years hospitalized with RSV bronchiolitis, admission and subsequently daily NW or ET aspirate with RSV culture (\log_{10} PFU/mL) Exclusion criteria: ID, CF, reactive airways disease or asthma, poorly controlled CHD, renal failure, ventilator dependency at the enrollment, life expectancy < 6 mo, apnea without evidence of LRTI, IVIG therapy in the preceding 2 months, Ribavirin in the preceding month	77 patients, 33/77 healthy infants, 18/77 infants with CHD \pm BPD \pm prematurity (< 32 w), 11/77 infants with BPD \pm prematurity, 15/77 infants with prematurity	Specified respiratory illness score	No correlation between severity of illness judged by respiratory illness score and RSV titer ($p > 0.05$)
[15]	Cross-sectional study on children ≤ 2 years hospitalized for LRTI, NPA at admission with RSV load expressed by PCR-CT values (inverse linear relationship between viral load and CT-values) Exclusion criteria: NA	120 patients, 98/120 viral positive NPA, 58/98 with RSV infection, 27/98 with RV infection, 11/98 RSV+RV infection	LOS	Trend, although not significant, in correlation between RSV CT-value and LOS ($p = 0.05$)
[20]	Cross-sectional study on infants ≤ 1 year hospitalized for bronchiolitis, NPW at admission with RSV load (copies/mL) Exclusion criteria: BPD, CHD, CF, bronchiolitis obliterans, Bordetella pertussis infection, previous macrolides treatment	110 patients, 56/110 samples analyzed with RSV load by quantitative RT-PCR	Duration of wheezing, LOS Duration of oxygen therapy	No correlation between RSV load and duration of wheezing ($p > 0.05$), LOS ($p > 0.05$), duration of oxygen therapy ($p > 0.05$)
[21]	Prospective study on children (age not specified) hospitalized for LRTI (pneumonia, bronchitis, bronchiolitis), NPA at admission with RSV load (copies/mL) No exclusion criteria	387 patients, 160/387 patients with bronchiolitis, RSV 1 st pathogen in bronchiolitis (data about frequency NA)	Specified criteria for severity disease definition	No correlation between RSV load and severity disease defined by specified clinical signs ($p > 0.05$)
[26]	Prospective study on children < 2 years hospitalized for RSV bronchiolitis, NP swab at admission and daily during hospitalization, then weekly until no more RSV detection, with RSV viral load (\log_{10} RNA copies/mL) Exclusion criteria: Use of Palivizumab, drop-out at follow-up	38 patients, 247 samples	Need for PICU, Need for intubation, LOS	No significant differences in RSV load and need for PICU ($p > 0.05$), nor for intubation ($p > 0.05$), nor prolonged LOS ($p > 0.05$)

Table S3. Studies finding no correlation between RSV load and bronchiolitis severity.

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