# Supplementary information

# Biomarkers FOR DISEASE SEVERITY IN CHILDREN INFECTED WITh Respiratory syncytial virus (RSV): a systematic literature review

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## Supplementary methods

### **Quality of evidence and assessment of risk of bias**

The grading is done by assigning low, moderate, and high risk of bias for each parameter, according to GRADE guidelines[3]. The interpretation of the risk of bias grading is done for each parameter: 1) inclusion of control population, 2) appropriate measurement of both exposure and outcome, 3) adequate control confounding, and 4) follow-up.

Specifics of the grading is as follows. For the inclusion of control population: “low” if a healthy control group is included in the study, “medium” if unclear, and “high” if no control group is included in the study. For the appropriate measurement of exposure and outcome: “low” if the study used a clinical severity score or assessed with a validated method; “medium” if the study mentioned the severity details and the laboratory outcomes however with insufficient details; “high” if severe RSV infection is defined by the laboratory outcomes. For the adequately control confounding: “low” if more than one confounding factor is mentioned clearly; “medium” if only one confounding factor is mentioned, or it unclear about the confounders; “high” if confounding factors are not mentioned. For the follow-up: “low” if the study includes no obvious to loss of follow up or no follow up; “medium” if there is no mention of the follow-up; “high” if there is a loss to follow-up.

## Supplementary figures and tables



**Supplementary figure 1:** PRISMA diagram[1]. Overview of data selection is represented.



**Supplementary figure 2:** Risk of bias assessment. The risk of bias assessment was performed according to the PRISMA guidelines. Inclusion of control population (controls), adequately control confounding (confounding), loss-of follow-up of patients (follow-up) and, appropriate measurement of both exposure and outcome measured were graded as high (coloured in red), moderate (coloured in yellow) and low (coloured in green) risk of bias.



**Supplementary figure 3**: Overview of comparison of transcriptomics studies assessing the association between severe RSV disease versus mild RSV disease in infants. Two whole genome transcriptomics studies comparing gene expression profiles in whole blood or PBMCs of severe and mild RSV disease in infants were identified[2,3]. Eighteen genes overlapped between the listed datasets compromising the genes that are differentially expressed in blood samples of infants with severe RSV disease versus mild RSV disease. The genes listed in green are reported to be upregulated genes, and the genes listed in red are reported to be downregulated genes.

**Supplementary table 1**: Summary of studies assessing the association of various genetic polymorphisms and RSV disease severity. Only the genes that are linked with severe RSV or with conflicted data (association or no association) were shown. Genes are represented according to the HUGO Gene Nomenclature Committee. N/A is noted when polymorphism is not associated with amino acid and codon change.

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| ***Gene* symbol****(protein)** | **Name of the polymorphism (rsnumber)** | **Amino acid changeCodon change** | **Publication** | **Number of patients** | **Association** | **Severity parameters** |
| *CNR2* (CB2) | rs35761398 | Gln63ArgQ [CAA] > R [CGG] | Tahamtan *et al*, 2017[4] | 45 inpatients; 38 outpatients | Increased risk of hospitalisation was associated with carrying Q allele (OR = 2.148; 95% CI = 1.092–4.224; p = 0.026) | Hospitalisation |
| *CX3CR1* (CX3CR1) | rs3732378 | Thr280MetT [ACG] > M [ATG] | Amanatidou *et al*, 2006[5] | 82 infants hospitalised for RSV-induced bronchiolitis; 120 healthy controls | Carrying M allele was associated with lower oxygen saturation (OR = 3.8; 95% CI = 1.5–9.8; p = 0.006) and with the need for supplemental oxygen (OR= 2.2; 95% CI = 1.1–4.4; p = 0.019) | Oxygen saturation; need for supplemental oxygen |
| *IFNG* (IFNγ) | rs3138557 (CA microsatellite) | N/A | Huang *et al*, 2014[6] | 218 infants hospitalised with RSV bronchiolitis and 303 healthy controls | CA12+/CA12+ and CA12+/CA12- genotypes were associated with RSV severity [2.84 ± 0.40 (mean ± SD) and 2.95 ± 0.44 (mean ± SD), respectively] | RSV severity score: Respiratory rates; oxygen saturation; wheezing; rales; retractions |
| *IFNG* (IFNγ) | rs2430561 | N/A | Gentile *et al*, 2003[7] | 77 infants hospitalised due to RSV-infection; 107 healthy controls | *IFNG* genotypes were associated with severity score (β = 0.22; r2 = 0.05; p = 0.061), duration of ICU stay (β = 0.24; r2 = 0.06; p = 0.021), and frequency of the otitis media (β = -0.85; r2 = 0.06; χ2=4.3; p = 0.04) | Severity score (respiratory rate, oxygen saturation, wheeze, retraction); duration of ICU stay; frequency of otitis media |
| *IL10* (IL-10) | rs1800896,rs1800871,rs1800872 | N/AN/AN/A | Gentile *et al*, 2003[7] | 77 infants hospitalised due to RSV-infection; 107 healthy controls | *IL-10* genotypes were associated with frequency of diagnosed pneumonia (β = -1.78; r2 = 0.07; χ 2 = 5.6; p = 0.02) and physical exam score (β = 0.27; r2 = 0.07; χ2 = 2.69; p = 0.009) | Frequency of pneumonia; physical exam score |
| *IL10* (IL-10) | rs1800896;rs1800890 | N/AN/A | Wilson *et al*, 2005[8] | 580 infants with RSV bronchiolitis; 580 healthy controls | For rs1800896 (IL-10 -1117), allele G (OR = 1.68; p = 0.004 and genotype GG (OR = 2.06; p = 0.006), and for rs1800890 (IL10 -3585), allele A (OR = 1.58; p = 0.01) and genotype AA (OR = 1.91; p = 0.01) were associated with the need of mechanical ventilation | Need of mechanical ventilation |
| *IL1RL1* (IL1RL1) | rs1921622 | N/A | Faber *et al*, 2012[9] | 465 infants with RSV bronchiolitis; 930 control subjects | Overrepresentation of G allele (p=0.011) and genotype (GG and/or GA) level (p=0.04) were associated with need for mechanical ventilation | Need for mechanical ventilation |
| *IL6* (IL-6) | rs1800795 | N/A | Gentile *et al*, 2003[7] | 77 infants hospitalised due to RSV-infection; 107 healthy controls | *IL-6* genotypes were associated with duration of oxygen supplementation (β = -0.22; r2 = 0.05; p = 0.025) and hospital stay (β = -0.27; r2 = 0.07; p = 0.009) | Duration of supplemental oxygen; hospital stay |
| *CXCL8* (IL-8) | rs4073 | N/A | Hull *et al*, 2000[10] | 117 nuclear families with a child requiring hospitalisation due to RSV infection | Likelihood transmission of the IL-8-251A allele was associated with need for oxygen therapy (%95 CI: 54 – 76, p = 0.011) and duration of supplemental oxygen (%95 CI: 58 to 84, p = 0.05) | Need for oxygen therapy; duration of supplemental oxygen |
| *SFTPA2* (SP-A2) | rs1059046 | Thr9AsnT [ACC] > S [AGC] | El Saleeby *et al*, 2010[11] | 291 RSV-induced infections | Homozygosity of 1A0 allele was found to be protective against hospitalisation (OR = 0.15, p = 0.001). Homozygous or heterozygous Asn 9 was associated with ICU admission (OR = 2.15; p = 0.022), require intubation (OR = 3.04; p = 0.005), and longer duration of hospitalisation (OR = 1.89; p = 0.02)  | Hospitalisation; ICU admission; need for intubation; duration of hospitalisation  |
| *SFTPD* (SP-D) | rs2243639 | Thr160AlaT [ACA] > A [GCA] | Ampuero *et al*, 2011[12] | 59 severe; 34 moderate; 25 mild RSV-infected infants; 104 blood donors as control subjects | Thr160 allele was associated with RSV severity (OR = 2.33, p = 0.015), and Ala160 allele was associated with milder RSV disease (OR = 0.42, p = 0.015) | Clinical severity scoring: Length of hospitalisation; ICU stay; need for supplemental oxygen; need for mechanical ventilation; duration of supplemental oxygen |
| *TGFB1* (TGF-β1) | rs1800470;rs1800471 | P (Pro) > L (Leu or Arg)P [CCG] > L [CTG];R (Arg) > P (Pro or Gln)R [CGG] > P [CCG] | Gentile *et al*, 2003[7] | 77 infants hospitalised due to RSV-infection; 107 healthy controls | *TGFB1* genotypes were associated with oxygen saturation at presentation (β = -0.29; r2 = 0.09; p = 0.008) | Oxygen saturation |
| *TIMP1* (TIMP1) | rs4898 | F (Phe) > F (Phe)F [TTT] > F [TTC] | Schuurhof *et al*, 2012[13] | 465 infants hospitalised due to RSV infection (81 of whom required mechanical ventilation); 930 control subjects | T-allele had higher frequency in males with need for mechanical ventilation (OR = 2.168; 95 % CI = 1.161 -4.049) | Need for mechanical ventilation |
| *TLR4* (TLR4) | rs4986790;rs4986791 | Asp299GlyD [GAT] > V [GTT];Thr399IleT [ACC] > I [ATC] | Mandelberg *et al*, 2006[14] | 21 ambulatory infants; 26 infants hospitalised on ward; 5 infants admitted to ICU | *TLR4* mutations were overrepresented in moderate and severe RSV compared to mild RSV disease (p < 0.005).  | Ambulatory infants (mild); Hospitalisation on ward (moderate); admission to ICU (severe) |
| *TLR4* (TLR4) | rs4986790;rs4986791 | Asp299GlyD [GAT] > V [GTT];Thr399IleT [ACC] > I [ATC] | Tal *et al*, 2004[15] | 82 infants with mild RSV bronchiolitis (ambulatory); 99 infants hospitalised with severe RSV bronchiolitis; 90 healthy controls | Asp299Gly (OR = 5.1; p = 0.014) and Thr399Ile (OR = 4.0; p = 0.01) are associated with hospitalisation. | Hospitalisation |
| *GC* (DBP) | rs7041 | D (Asp) > E (Glu)D [GAT] > E [GAG]) | Randolph *et al*, 2014[16] | 98 infants with severe RSV infection; 333 parents. Validation cohort - 465 infants hospitalised due to RSV bronchiolitis; 930 healthy controls | C allele was overrepresented in hospitalised infants (OR = 1.12; 95% CI = 1.02 – 1.4, p = 0.003) and infants with need of mechanical ventilation (OR = 1.57; 95% CI = 1.12 – 2.22, p = 0.009) | Hospitalisation; need for mechanical ventilation |

**Supplementary table 2**: Overview of studies assessing the association between various cytokine/chemokine levels in respiratory samples (*e.g.* nasal wash, nasopharyngeal aspirates, broncho-alveolar lavage [BAL]) and RSV disease severity. “(-)” indicates negative association, “(+)” indicates a positive association, and “(na)” indicates no association observed by the corresponding publication.

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| **Cytokine/chemokine** | **Publication** | **Association** | **Age group** | **Number of patients** | **Severity parameters** |
| RANTES (CCL5) | Thwaites *et al*, 2018[17] | (-) | 2 weeks to 24 months | 12 infants with severe RSV, 18 infants with moderate RSV disease | Need for mechanical ventilation (severe); hospitalisation without the need for mechanical ventilation (moderate) |
| Vieira *et al*, 2010[18] | (na) | < 3 months | 30 RSV LRTI | Clinical severity score (Respiratory rate; wheezing; oxygen saturation; accessory muscle recruitment); duration of oxygen therapy; duration of mechanical ventilation; length of hospital stay |
| Tabarani *et al*, 2013[19] | (na) | < 5 years | 268 infants with mild RSV; 503 infants with moderate RSV; 80 infants with severe RSV; 126 healthy controls | Need for mechanical ventilation (severe); hospitalisation without the need for mechanical ventilation (moderate); not requiring hospitalisation (mild) |
| RANTES (CCL5): IL-10 ratio | Hornsleth *et al*, 2001[20] | (+) | < 9 months | 25 infants with severe RSV, 21 infants with mild RSV disease | Clinical severity score: Duration of hospital stay; need of respiratory support |
| EGF | Tabarani *et al*, 2013[19] | (+) | < 5 years | 268 infants with mild RSV; 503 infants with moderate RSV; 80 infants with severe RSV; 126 healthy controls | Need for mechanical ventilation (severe); hospitalisation without the need for mechanical ventilation (moderate); not requiring hospitalisation (mild) |
| FGF-β | Garcia *et al*, 2011[21] | (-) | < 24 months | 19 infants with RSV infection; 18 infants with HRV-induced LRTI | Length of stay |
| HGF | Tabarani *et al*, 2013[19] | (+) | < 5 years | 268 infants with mild RSV; 503 infants with moderate RSV; 80 infants with severe RSV; 126 healthy controls | Need for mechanical ventilation (severe); hospitalisation without the need for mechanical ventilation (moderate); not requiring hospitalisation (mild) |
| IFN-α | Tabarani *et al*, 2013[19] | (+) | < 5 years | 268 infants with mild RSV; 503 infants with moderate RSV; 80 infants with severe RSV; 126 healthy controls | Need for mechanical ventilation (severe); hospitalisation without the need for mechanical ventilation (moderate); not requiring hospitalisation (mild) |
| IFN-γ | Bennett *et al*, 2007[22] | (-) | < 24 months | 101 infants hospitalised due to bronchiolitis | Duration of supplemental oxygen therapy |
| Bont *et al* 2001[23] | (-) | < 13 months | 17 infants with RSV LRTI requiring mechanical ventilation; 43 infants with RSV LRTI with no mechanical ventilation | Need for mechanical ventilation |
| Garcia *et al*, 2011[21]  | (-) | < 24 months | 19 infants with RSV infection; 18 infants with HRV-induced LRTI  | Length of hospitalisation; duration of supplemental oxygen |
| Legg *et al*, 2003[24] | (-) | [mean days (sd)] LRTI group: 147 (84); URTI group: 219 (125) | 9 RSV LRTI; 19 RSV URTI | Acute URTI versus acute bronchiolitis |
| Semple *et al*, 2007[25] | (-) | < 24 months | 56 infants with severe RSV; 114 infants with moderate RSV; 27 infants with mild RSV | Hospitalised infants requiring mechanical ventilation (severe); Hospitalised infants need of supplemental oxygen; hospitalised infants not requiring oxygen (mild) |
| Thwaites *et al*, 2018[17] | (-) | 2 weeks to 24 months | 12 infants with severe RSV, 18 infants with moderate RSV disease | Need for mechanical ventilation (severe); hospitalisation without the need for mechanical ventilation (moderate) |
| Assefa *et al*, 2011[26] | (na) | < 6 months | 13 term infants hospitalised due to RSV bronchiolitis; 11 preterm infants hospitalised due to RSV bronchiolitis | Clinical severity score (respiratory rate; wheezing, retractions, general appearance); peripheral oxygen saturation; duration of oxygen supplement |
| Bermejo-Martin *et al*, 2007[27] | (na) | < 24 months | 22 severe RSV LRTI; 22 healthy controls | M-WCAS scoring system |
| IL-10 | Vieira *et al*, 2010[18] | (+) | < 3 months | 30 RSV LRTI | Clinical severity score: Respiratory rate; wheezing; oxygen saturation; accessory muscle recruitment |
| Bennett *et al*, 2007[22] | (-) | < 24 months | 101 infants hospitalised due to bronchiolitis | The duration of supplemental oxygen therapy |
| Bont *et al*, 2001[23] | (na) | < 13 months | 17 infants with RSV LRTI requiring mechanical ventilation; 43 infants with RSV LRTI with no mechanical ventilation | Need for mechanical ventilation |
| Garcia *et al*, 2011[21]  | (na) | < 24 months | 19 infants with RSV infection; 18 infants with HRV-induced LRTI  | Clinical severity score (Oxygen saturation; respiratory rate; presence of retractions; wheezing; need for intravenous fluid and general condition); length of hospitalisation; duration of supplemental oxygen; admission to PICU; mechanical intubation  |
| Welliver *et al*, 2007[28] | (na) | < 12 months | 45 RSV-infected infants (6 URTI, 30 bronchiolitis, 9 fatal bronchiolitis); 47 Flu-infected infants (24 URTI, 12 bronchiolitis, 11 fatal bronchiolitis) | URTI, LRTI, oxygen saturation |
| Legg *et al*, 2003[24] | (na) | [mean days (sd)] LRTI group: 147 (84); URTI group: 219 (125) | 9 RSV LRTI; 19 RSV URTI | Acute URTI versus acute bronchiolitis |
| IL-17A | Christiaansen *et al*, 2016[29] | (-) | < 12 months | 23 RSV-infected infants; 17 healthy controls | Difficulty in breathing; retractions |
| IL-1R-α | Tabarani *et al*, 2013[19] | (+) | < 5 years | 268 infants with mild RSV; 503 infants with moderate RSV; 80 infants with severe RSV; 126 healthy controls | Need for mechanical ventilation (severe); hospitalisation without the need for mechanical ventilation (moderate); not requiring hospitalisation (mild) |
| Bermejo-Martin *et al*, 2008[30] | (-) | < 24 months | 14 infants with RSV/A infection; 8 infants with RSV/B infection; 11 infants with co-infection; 27 healthy controls | M-WCAS scoring system |
| IL–1β | Tabarani *et al*, 2013[19] | (+) | < 5 years | 268 infants with mild RSV; 503 infants with moderate RSV; 80 infants with severe RSV; 126 healthy controls | Need for mechanical ventilation (severe); hospitalisation without the need for mechanical ventilation (moderate); not requiring hospitalisation (mild) |
| Diaz *et al*, 2013[31] | (+) | < 12 months | 49 RSV bronchiolitis (25of whom have severe bronchiolitis) | Clinical severity score (days of hospitalisation; need for oxygen; maximum oxygen fraction; outpatients; ICU stay) |
| Christiaansen *et al*, 2016[29] | (-) | < 12 months | 23 RSV-infected infants; 17 healthy controls | Difficulty in breathing; retractions |
| Bennett *et al*, 2007[22] | (na) | < 24 months | 101 infants hospitalised due to bronchiolitis | Need of hospitalisation; duration of IVF therapy; the duration of supplemental oxygen therapy |
| Bermejo-Martin *et al*, 2007[27] | (na) | < 24 months | 22 severe RSV LRTI; 22 healthy controls | M-WCAS scoring system |
| Garcia *et al*, 2011[21]  | (na) | < 24 months | 19 infants with RSV infection; 18 infants with HRV-induced LRTI  | Clinical severity score (oxygen saturation; respiratory rate; presence of retractions; wheezing; need for intravenous fluid and general condition); length of hospitalisation; duration of supplemental oxygen; admission to PICU; mechanical intubation |
| Welliver *et al*, 2007[28] | (na) | < 12 months | 45 RSV (6 URTI, 30 bronchiolitis, 9 fatal bronchiolitis); 47 Flu (24 URTI, 12 bronchiolitis, 11 fatal bronchiolitis) | URTI versus LRTI, oxygen saturation |
| IL-2 | Tabarani *et al*, 2013[19] | (+) | < 5 years | 268 infants with mild RSV; 503 infants with moderate RSV; 80 infants with severe RSV; 126 healthy controls | Need for mechanical ventilation (severe); hospitalisation without the need for mechanical ventilation (moderate); not requiring hospitalisation (mild) |
| Bennett *et al*, 2007[22] | (na) | < 24 months | 101 infants hospitalised due to bronchiolitis | Need of hospitalisation; duration of IVF therapy; the duration of supplemental oxygen therapy |
| Bermejo-Martin *et al*, 2008[30] | (na) | < 24 months | 14 infants with RSV/A infection; 8 infants with RSV/B infection; 11 infants with co-infection; 27 healthy controls |  M-WCAS scoring system; oxygen saturation |
| Welliver *et al*, 2007[28] | (na) | < 12 months | 45 RSV (6 URTI, 30 bronchiolitis, 9 fatal bronchiolitis); 47 Flu (24 URTI, 12 bronchiolitis, 11 fatal bronchiolitis) | URTI versus LRTI, oxygen saturation |
| Giugno *et al*, 2004[32] | (na) | < 24 months | 62 infants hospitalised due to RSV infection (26 mild, 24 moderate, 5 severe, 7 normal) | Oxygen saturation; modified clinical score system (duration of oxygen supplement, length of hospital stay, and need for mechanical ventilation) |
| IL-2R | Tabarani *et al*, 2013[19] | (+) | < 5 years | 268 infants with mild RSV; 503 infants with moderate RSV; 80 infants with severe RSV; 126 healthy controls | Need for mechanical ventilation (severe); hospitalisation without the need for mechanical ventilation (moderate); not requiring hospitalisation (mild) |
| IL-23 | Christiaansen *et al*, 2016[29] | (-) | < 12 months | 23 RSV-infected infants; 17 control subjects | Difficulty in breathing; retractions |
| IL-4 | Legg *et al*, 2003[24] | (+) | [mean days (sd)]LRTI group: 147 (84); URTI group: 219 (125) | 9 RSV LRTI; 19 RSV URTI | Acute URTI versus acute bronchiolitis |
| Assefa *et al*, 2011[26] | (na) | < 6 months | 13 term infants hospitalised due to RSV bronchiolitis; 11 preterm infants hospitalised due to RSV bronchiolitis | Clinical severity score (respiratory rate; wheezing, retractions, general appearance); peripheral oxygen saturation; duration of oxygen supplement |
| Bennett *et al*, 2007[22] | (na) | < 24 months | 101 infants hospitalised due to bronchiolitis | Need of hospitalisation; duration of IVF therapy; the duration of supplemental oxygen therapy |
| Welliver *et al*, 2007[28] | (na) | < 12 months | 45 RSV (6 URTI, 30 bronchiolitis, 9 fatal bronchiolitis); 47 Flu (24 URTI, 12 bronchiolitis, 11 fatal bronchiolitis) | URTI, LRTI, oxygen saturation |
| IL-4:IFN-γ ratio | Legg *et al*, 2003[24] | (+) | [mean days (sd)]LRTI group: 147 (84); URTI group: 219 (125) | 9 RSV LRTI; 19 RSV URTI | Acute URTI versus acute bronchiolitis |
| IL-6 | Tabarani *et al*, 2013[19] | (+) | < 5 years | 268 infants with mild RSV; 503 infants with moderate RSV; 80 infants with severe RSV; 126 healthy controls | Need for mechanical ventilation (severe); hospitalisation without the need for mechanical ventilation (moderate); not requiring hospitalisation (mild) |
| Brand *et al* 2013[33] | (+) | < 24 months | 52 infants with RSV infection (11 mild, 22 moderate, 19 severe) | Need for mechanical ventilation (severe); need for supplemental oxygen and/or nasopharyngeal feeding (moderate); no need for supportive treatment (mild) |
| Diaz *et al*, 2013[31] | (+) | < 12 months | 49 RSV bronchiolitis (25 severe bronchiolitis) | Clinical severity score (days of hospitalisation, need for oxygen, maximum oxygen fraction delivered during hospitalisation, outpatients, and ICU stay) |
| Moreno-Solis *et al*, 2015[34] | (+) | 1-12 months | 45 RSV-AB; 27 control subjects | Need for oxygen supplement |
| Bennett *et al*, 2007[22] | (-) | < 24 months | 101 infants hospitalised due to bronchiolitis | Duration of supplemental oxygen therapy |
| Garcia *et al*, 2011[21]  | (na) | < 24 months | 19 infants with RSV infection; 18 infants with HRV-induced LRTI  | Clinical severity score (oxygen saturation; respiratory rate; presence of retractions; wheezing; need for intravenous fluid and general condition); length of hospitalisation; duration of supplemental oxygen; admission to PICU; mechanical intubation |
| Welliver *et al*, 2007 | (na) | < 12 months | 45 RSV (6 URTI, 30 bronchiolitis, 9 fatal bronchiolitis); 47 Flu (24 URTI, 12 bronchiolitis, 11 fatal bronchiolitis) | URTI versus LRTI, oxygen saturation |
| Vieira *et al*, 2010[18] | (na) | < 3 months | 30 RSV LRTI | Clinical severity score (Respiratory rate; wheezing; oxygen saturation; accessory muscle recruitment); duration of oxygen therapy; duration of mechanical ventilation; length of hospital stay |
| IL-6:TNF-α ratio | Hornsleth *et al*, 1998[35] | (-) | < 9 months | 46 infants with RSV infection (25 severe, 21 mild) | Clinical severity score: Duration of hospital stay; the need of respiratory support |
| IL-7 | Tabarani *et al*, 2013[19] | (+) | < 5 years | 268 infants with mild RSV; 503 infants with moderate RSV; 80 infants with severe RSV; 126 healthy controls | Need for mechanical ventilation (severe); hospitalisation without the need for mechanical ventilation (moderate); not requiring hospitalisation (mild) |
| Welliver *et al*, 2007[28] | (na) | < 12 months | 45 RSV (6 URTI, 30 bronchiolitis, 9 fatal bronchiolitis); 47 Flu (24 URTI, 12 bronchiolitis, 11 fatal bronchiolitis) | URTI, LRTI, oxygen saturation  |
| IL-8 | Assefa *et al*, 2011[26] | (+) | < 6 months | 13 term infants hospitalised due to RSV bronchiolitis; 11 preterm infants hospitalised due to RSV bronchiolitis | Clinical severity score (respiratory rate; wheezing, retractions, general appearance) |
| Diaz *et al*, 2013[31] | (+) | < 12 months | 49 RSV bronchiolitis (25of whom have severe bronchiolitis) | Clinical severity score (days of hospitalisation; need for oxygen; maximum oxygen fraction; outpatients; ICU stay) |
| Tabarani *et al*, 2013[19] | (+) | < 5 years | 268 infants mild RSV; 503 infants with moderate RSV; 80 infants with severe RSV; 126 controls | Need for mechanical ventilation (severe); hospitalisation without the need for mechanical ventilation (moderate); not requiring hospitalisation (mild) |
| Bennett *et al*, 2007[22] | (-) | < 24 months | 101 infants hospitalised due to bronchiolitis | Duration of supplemental oxygen therapy |
| Welliver *et al*, 2007[28] | (na) | < 12 months | 45 RSV (6 URTI, 30 bronchiolitis, 9 fatal bronchiolitis); 47 Flu (24 URTI, 12 bronchiolitis, 11 fatal bronchiolitis) | URTI versus LRTI, oxygen saturation |
| Bermejo-Martin *et al*, 2007[27] | (na) | < 24 months | 22 severe RSV LRTI; 22 healthy controls | M-WCAS scoring system |
| IL-8:RANTES ratio | Hornsleth *et al*, 2001[20] | (+) | < 9 months | 46 infants with RSV infection (25 severe, 21 mild) | Clinical severity score: Respiratory rate; alveolar or peribranchial infiltration; duration of hospital stay; the need of respiratory support |
| MCP-1 | Tabarani *et al*, 2013[19] | (+) | < 5 years | 268 infants with mild RSV; 503 infants with moderate RSV; 80 infants with severe RSV; 126 healthy controls | Need for mechanical ventilation (severe); hospitalisation without the need for mechanical ventilation (moderate); not requiring hospitalisation (mild) |
| Bennett *et al*, 2007[22] | (na) | < 24 months | 101 infants hospitalised due to bronchiolitis | Need of hospitalisation; duration of IVF therapy; the duration of supplemental oxygen therapy |
| Garcia *et al*, 2011[21]  | (na) | < 24 months | 19 infants with RSV infection; 18 infants with HRV-induced LRTI  | Clinical severity score (Oxygen saturation; respiratory rate; presence of retractions; wheezing; need for intravenous fluid and general condition); length of hospitalisation; duration of supplemental oxygen; admission to ICU; mechanical intubation |
| MIP-1α | Tabarani *et al*, 2013[19] | (+) | < 5 years | 268 infants with mild RSV; 503 infants with moderate RSV; 80 infants with severe RSV; 126 healthy controls | Need for mechanical ventilation (severe); hospitalisation without the need for mechanical ventilation (moderate); not requiring hospitalisation (mild) |
| MIP-1β | Tabarani *et al*, 2013[19] | (+) | < 5 years | 268 infants with mild RSV; 503 infants with moderate RSV; 80 infants with severe RSV; 126 healthy controls | Need for mechanical ventilation (severe); hospitalisation without the need for mechanical ventilation (moderate); not requiring hospitalisation (mild) |
| Moreno-Solis *et al*, 2015[34] | (+) | 1-12 months | 45 RSV-AB; 27 healthy controls | Need for oxygen supplement |
| Bennett *et al*, 2007[22] | (-) | < 24 months | 101 infants hospitalised due to bronchiolitis | Duration of supplemental oxygen therapy |
| PDGF-ββ | Garcia *et al*, 2011[21]  | (-) | < 24 months | 19 infants with RSV infection; 18 infants with HRV-induced LRTI  | Clinical severity score (Oxygen saturation; respiratory rate; presence of retractions; wheezing; need for intravenous fluid and general condition); length of stay; days of supplemental oxygen |
| sICAM-1 | Vieira *et al*, 2010[18] | (+) | < 3 months | 30 RSV LRTI | Clinical severity score: Respiratory rate; wheezing; oxygen saturation; accessory muscle recruitment |
| SP | Semple *et al*, 2007[25] | (-) | < 24 months | 56 infants with severe RSV; 114 infants with moderate RSV; 27 infants with mild RSV | Hospitalised infants requiring mechanical ventilation (severe); Hospitalised infants need of supplemental oxygen; hospitalised infants not requiring oxygen (mild) |
| TNF-R1:RANTES ratio | Hornsleth *et al*, 2001[20] | (+) | < 9 months | 46 infants with RSV infection (25 severe, 21 mild) | Clinical severity score: Respiratory rate; alveolar or peribronchial infiltration; duration of hospital stay; the need of respiratory support |
| TNF-α | Tabarani *et al*, 2013[19] | (+) | < 5 years | 268 infants with mild RSV; 503 infants with moderate RSV; 80 infants with severe RSV; 126 healthy controls | Need for mechanical ventilation (severe); hospitalisation without the need for mechanical ventilation (moderate); not requiring hospitalisation (mild) |
| Bennett *et al*, 2007[22] | (na) | < 24 months | 101 infants hospitalised due to bronchiolitis | Need of hospitalisation; duration of IVF therapy; the duration of supplemental oxygen therapy |
| Bermejo-Martin *et al*, 2007[27] | (na) | < 24 months | 22 severe RSV LRTI; 22 control infants | M-WCAS scoring system |
| Garcia *et al*, 2011[21]  | (na) | < 24 months | 19 infants with RSV infection; 18 infants with HRV-induced LRTI  | Clinical severity score (oxygen saturation; respiratory rate; presence of retractions; wheezing; need for intravenous fluid and general condition); length of hospitalisation; duration of supplemental oxygen; admission to PICU; mechanical intubation |
| Vieira *et al*, 2010[18] | (na) | < 3 months | 30 RSV LRTI | Clinical severity score (Respiratory rate; wheezing; oxygen saturation; accessory muscle recruitment); duration of oxygen therapy; duration of mechanical ventilation; length of hospital stay |
| Christiaansen *et al*, 2016[29] | (na) | < 12 months | 23 RSV-infected infants; 17 control subjects | Difficulty in breathing; retractions |

**Supplementary table 3**: Overview of studies assessing the association between respiratory microbiome and RSV disease severity. “(-)” indicates negative association, “(+)” indicates a positive association observed by the corresponding publication.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Bacteria** | **Publication** | **Association** | **Age group** | **Number of patients** | **Severity parameters** |
| Gram negative | *Moraxella catarrhalis* | Teo *et al*., 2015[36] | (+) | < 12 months | 234 infants | LRTI versus URTI; fever in LRTI  |
| Teo *et al*., 2018[37] | (+) | *< 5 years* | 244 infants | LRTI versus URTI |
| *Suárez-Arrabal et al., 2015*[38] | (+) | < 2 years | 136 RSV bronchiolitis; 23 healthy controls | Duration of oxygen supplementation |
| *Haemophilus influenzae* | Teo *et al*., 2015[36] | (+) | < 12 months | 234 infants | LRTI versus URTI |
| *Teo et al., 2018*[37] | (+) | *< 5 years* | 244 infants | LRTI versus URTI  |
| *Jiang et al., 2015*[39] | (+) | < 2 years | 608 bronchiolitis cases (252 RSV; 188 positives for potentially pathogenic bacteria or PPB) | Length of hospitalization |
| *Suárez-Arrabal et al., 2015*[38] | (+) | < 2 years | 136 RSV bronchiolitis; 23 healthy controls | Duration of oxygen supplementation |
| *de Steenhuijsen Piters et al., 2016*[40] | (+) | < 24 months | 84 RSV inpatients and 22 RSV outpatients, 26 control subjects | Hospitalisation |
| *Ederveen et al., 2018*[41] | (+) | < 6 months | 54 infants infected with RSV (9 mild, 27 moderate, 18 severe); 21 healthy controls | Need for mechanical ventilation (severe); need for supplemental oxygen (moderate); no hypoxemia (mild) |
| Gram positive | *Staphylacoccus aureus* | *de Steenhuijsen Piters et al., 2016*[40] | (-) | < 24 months | 84 RSV inpatients and 22 RSV outpatients, 26 healthy controls | Hospitalisation |
| *Streptococcus pneumoniae* | Teo *et al*., 2015[36] | (+) | < 12 months | 234 infants | LRTI versus URTI |
| *Teo et al., 2018*[37] | (+) | *< 5 years* | 244 infants | LRTI versus URTI  |
| *de Steenhuijsen Piters et al., 2016*[40] | (+) | < 24 months | 84 RSV inpatients and 22 RSV outpatients, 26 control subjects | Hospitalisation |
| *Brealey et al., 2018*[42] | (+) | < 24 months | 58 infants hospitalised due to respiratory infection | M-WCAS scoring system |

**Supplementary table 4**: Overview of studies assessing the association between various cytokine/chemokine levels in blood and RSV disease severity. “(-)” indicates negative association, “(+)” indicates a positive association, and “(na)” indicates no association observed by the corresponding publication.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Cytokine/chemokine** | **Publication** | **Association** | **Age group** | **Number of patients** | **Severity parameters** |
| RANTES | *Brand et al, 2013*[33] | (-) | < 24 months | 52 infants with RSV infection (19 severe, 22 moderate, 11 mild) | Need for mechanical ventilation (severe); need for supplemental oxygen (moderate); no need for supportive treatment (mild) |
| *Vieira et al, 2010[47]* | (na) | < 3 months | 30 RSV LRTI | Clinical severity score (Respiratory rate; wheezing; oxygen saturation; accessory muscle recruitment); duration of oxygen therapy; duration of mechanical ventilation; length of hospital stay |
| G-CSF | *Brand et al, 2013*[33] | (+) | < 24 months | 52 infants with RSV infection (19 severe, 22 moderate, 11 mild) | Need for mechanical ventilation (severe); need for supplemental oxygen (moderate); no need for supportive treatment (mild) |
| IFN-γ | *Bendelja et al, 2000*[43] | (+) | 3 weeks - 24 months | 30 infants with RSV (6 pneumonia, 17 bronchiolitis, 7 URTI); 10 healthy controls | Pneumonia; bronchiolitis; URTI |
| *Aberle et al, 1999*[44] | (-) | 3-44 weeks | 20 infants hospitalised due to RSV LRTI; 6 healthy controls | Need for supplemental oxygen |
| *Bont et al, 1999*[45] | (-) | < 13 months | 14 infants with severe RSV bronchiolitis; 36 infants with mild-moderate RSV bronchiolitis; 27 healthy controls | Need for mechanical ventilation (severe); other hospitalised infants (mild-moderate) |
| *Chen et al, 2002*[50] | (-)\* | 2-24 months | Cohort 1: 44 infants hospitalised due to RSV infection. Cohort 2: 41 non-RSV LRTI; 30 healthy controls | Severe group: Oxygen saturation lower than 90%, arterial oxygen partial pressure lower than 60mmHg, need for oxygen supplementation, mechanical ventilation; others are considered as mild group.\*Decrease in IFN-γ in CD3+CD8+ cells were observed. No changes were observed in CD3+CD8- cells and in whole blood.  |
| *Pinto et al, 2006*[46] | (-) | < 12 months | 21 inpatients (severe RSV); 21 outpatients (mild RSV); 21 healthy controls | Hospitalised infants (severe group) were scored with Tal clinical severity score and compared with the ambulatory infants (mild group). |
| *Fernández et al, 2005*[47] | (na) | < 12 months | 196 infants with RSV infection | Hypoxia (disease severity based on length of supplementary oxygen therapy); non-hypoxia |
| *Brandenburg et al, 2000*[48] | (na) | < 6 months | 111 hospitalised infants (50 confirmed severe RSV infection, 45 mild infection) | Severe infection: pCO2 > 6,6kPa, oxygen saturation < 90%, or need for mechanical ventilation; mild infection; other hospitalised infants |
| *Larrañaga et al, 2009*[49] | (na) | < 6 months | 75 infants with RSV infection (37 severe, 38 moderate); 24 healthy controls | Clinical severity score: Hospitalisation; need for supplemental oxygen; no maximal FIO2 (%) |
| *Moreno-Solis et al, 2015*[55] | (na) | 1-12 months | 45 RSV-AB; 27 healthy controls | Clinical severity score; need for supplemental oxygen |
| IL-10 | *Fernández et al, 2005*[47] | (+) | < 12 months | 196 infants with RSV infection | Hypoxia (disease severity based on length of supplementary oxygen therapy); non-hypoxia |
| *Bont et al, 2000*[51] | (na) | < 13 months | 30 infants with RSV bronchiolitis requiring mechanical ventilation | Duration of mechanical ventilation |
| *Brand et al, 2013*[33] | (na) | < 24 months | 52 infants with RSV infection (19 severe; 22 moderate; 11 mild) | Need for mechanical ventilation (severe); need for supplemental oxygen (moderate); no need for supportive treatment (mild) |
| *Brandenburg et al, 2000*[48] | (na) | < 6 months | 111 hospitalised infants (50 confirmed severe RSV infection, 45 mild infection) | Severe infection: pCO2 > 6,6kPa, oxygen saturation < 90%, or need for mechanical ventilation; mild infection: other hospitalised infants |
| *Pinto et al, 2006*[46] | (na) | < 12 months | 21 inpatients (severe RSV); 21 outpatients (mild RSV); 21 healthy controls | Hospitalised infants (severe group) were scored with Tal clinical severity score and compared with the ambulatory infants (mild group).  |
| *Larrañaga et al, 2009*[49] | (na) | < 6 months | 75 infants with RSV infection (37 severe, 38 moderate); 24 healthy controls | Clinical severity score: Hospitalisation; need for supplemental oxygen; no maximal FIO2 (%) |
| *Mella et al, 2013*[52] | (na) | < 24 months | 66 RSV LRTI (20 PICU patients, 46 floor patients); 14 healthy controls | Admission to PICU (severe); floor patients (mild-moderate). Severity is defined by a scoring system (length of hospitalization, need for supplemental oxygen, duration of supplemental oxygen) |
| *Vieira et al, 2010[47]* | (na) | < 3 months | 30 RSV LRTI | Clinical severity score (respiratory rate; wheezing; oxygen saturation; accessory muscle recruitment); duration of oxygen therapy; duration of mechanical ventilation; length of hospital stay |
| *Moreno-Solis et al, 2015*[55] | (na) | 1-12 months | 45 RSV-AB; 27 healthy controls | Clinical severity score; Need for oxygen supplement |
| IL-12 | *Bont et al, 2000*[51] | (-) | < 13 months | 30 infants with RSV bronchiolitis requiring mechanical ventilation | Duration of mechanical ventilation |
| *Pinto et al, 2006*[46] | (-) | < 12 months | 21 inpatients (severe RSV); 21 outpatients (mild RSV); 21 healthy controls | Hospitalised infants (severe group) were scored with Tal clinical severity score and compared with the ambulatory infants (mild group). |
| *Chen et al, 2002*[50] | (na) | 2-24 months | Cohort 1: 44 infants hospitalised due to RSV infection (26 LRTI, 18 pneumonia). Cohort 2: 41 non-RSV LRTI; 30 healthy controls | Severe group: Oxygen saturation lower than 90%; arterial oxygen partial pressure lower than 60mmHg; need for oxygen supplementation; mechanical ventilation; others are considered as mild group |
| IL-17 | *Larrañaga et al, 2009*[49] | (-) | < 6 months | 75 infants with RSV infection (37 severe, 38 moderate); 24 healthy controls | Clinical severity score: Hospitalisation; need for supplemental oxygen; no maximal FIO2 (%) |
| IL-4 | *Bendelja et al, 2000*[43] | (-) | 3 weeks - 24 months | 30 infants with RSV (6 pneumonia, 17 bronchiolitis, 7 URTI); 10 healthy controls | Pneumonia; bronchiolitis; URTI |
| *Bont et al, 1999*[45] | (-) | < 13 months | 14 infants with severe RSV bronchiolitis; 36 infants with mild-moderate RSV bronchiolitis; 27 healthy controls | Need for mechanical ventilation (severe); other hospitalised infants (mild-moderate) |
| *Chen et al, 2002*[50] | (na) | 2-24 months | Cohort 1: 44 infants hospitalised due to RSV infection (26 LRTI, 18 pneumonia). Cohort 2: 41 non-RSV LRTI; 30 healthy controls | Severe group: Oxygen saturation lower than 90%; arterial oxygen partial pressure lower than 60mmHg; need for oxygen supplementation; mechanical ventilation; others are considered as mild group |
| IL-4: IFN-γ ratio | *Legg et al, 2003*[24] | (+) | [mean days (sd)]LRTI group: 147 (84); URTI group: 219 (125) | 9 RSV LRTI; 19 RSV URTI | Acute URTI; acute bronchiolitis |
| *Chen et al, 2002*[50] | (na) | 2-24 months | Cohort 1: 44 infants hospitalised due to RSV infection (26 LRTI, 18 pneumonia). Cohort 2: 41 non-RSV LRTI; 30 healthy controls | Severe group: Oxygen saturation lower than 90%; arterial oxygen partial pressure lower than 60mmHg; need for oxygen supplementation; mechanical ventilation; others are considered as mild group |
| IL-6 | *Brand et al, 2013*[33] | (+) | < 24 months | 52 infants with RSV infection (19 severe, 22 moderate, 11 mild) | Need for mechanical ventilation (severe); need for supplemental oxygen (moderate); no need for supportive treatment (mild)  |
| *Brandenburg et al, 2000*[48] | (+) | < 6 months | 111 hospitalised infants (50 confirmed severe, 45 mild RSV infection) | Severe infection: pCO2 > 6,6kPa, oxygen saturation < 90%, or need for mechanical ventilation; mild infection: other hospitalised infants |
| *Diaz et al, 2013*[31] | (+) | < 12 months | 49 RSV bronchiolitis (25 severe bronchiolitis, 24 moderate) | Clinical severity score: days of hospitalisation, need for oxygen, maximum oxygen fraction delivered during hospitalisation, outpatients, and ICU stay |
| *Vieira et al, 2010**[47]* | (+) | < 3 months | 30 RSV LRTI | Clinical severity score (respiratory rate; wheezing; oxygen saturation; accessory muscle recruitment); duration of oxygen therapy; length of hospital stay |
| *Mella et al, 2013*[52] | (+)\* | < 24 months | 66 RSV LRTI (20 PICU patients, 46 floor patients); 14 healthy controls | Admission to PICU (severe); floor patients (mild-moderate). Severity is defined by a scoring system (length of hospitalization; the need for supplemental oxygen; duration of supplemental oxygen)\*IL-6 concentrations were positively correlated with the length of hospitalisation and length of PICU stay in PICU patients. |
| *Larrañaga et al, 2009*[49] | (na) | < 6 months | 75 infants with RSV infection (37 severe, 38 moderate); 24 healthy controls | Clinical severity score: Hospitalisation; need for supplemental oxygen; no maximal FIO2 (%) |
| *Hornsleth et al, 1998*[35] | (na) | < 4 years | 105 RSV LRTI | Clinical severity score: Respiratory rate; alveolar or peri bronchial infiltration; respiratory support; duration of hospitalisation |
| IL-6 | *Moreno-Solis et al, 2015*[55] | (na) | 1-12 months | 45 RSV-AB; 27 healthy controls | Clinical severity score; need for oxygen supplement |
| IL-8 | *Bermejo-Martin et al, 2007*[27] | (+) | < 24 months | 22 RSV LRTI; 22 healthy controls | Oxygen saturation (positive correlation with RSV severity) |
| *Bont et al, 1999*[53] | (+) | < 13 months | 14 infants with severe RSV bronchiolitis; 36 infants with mild-moderate RSV bronchiolitis; 27 healthy controls | Need for mechanical ventilation (severe); other hospitalised infants (mild-moderate) |
| *Brand et al, 2013*[33] | (+) | < 24 months | 52 infants with RSV infection (19 severe, 22 moderate, 11 mild) | Need for mechanical ventilation (severe); need for supplemental oxygen (moderate); no need for supportive treatment (mild) |
| *Brandenburg et al, 2000*[48] | (+) | < 6 months | 111 hospitalised infants (50 confirmed severe, 45 mild RSV infection) | Severe infection: pCO2 > 6,6kPa, oxygen saturation < 90% or need for mechanical ventilation; mild infection: other hospitalised infants |
| *Larrañaga et al, 2009*[49] | (+) | < 6 months | 75 infants with RSV infection (37 severe, 38 moderate); 24 healthy controls | Clinical severity score: Hospitalisation; need for supplemental oxygen; no maximal FIO2 (%) |
| *Mella et al, 2013*[52] | (+)\* | < 24 months | 66 RSV LRTI (20 PICU patients, 46 floor patients); 14 healthy controls | Admission to PICU (severe); floor patients (mild-moderate). Severity is defined by a scoring system (length of hospitalization; the need for supplemental oxygen; duration of supplemental oxygen)\*IL-8 concentrations were positively correlated with length of hospitalisation and higher severity score only in PICU patients. IL-8 production capacity is negatively associated with length of stay and RSV severity score |
| *Moreno-Solis et al, 2015*[55] | (na) | 1-12 months | 45 RSV-AB; 27 healthy controls | Clinical severity score; need for oxygen supplement |
| LL-37 | *Mansbach et al, 2017*[54] | (-) | < 12 months | 1005 enrolled infants | Intensive care stay; duration of hospital stay |
| MIP-1β | *Moreno-Solis et al, 2015*[55] | (-) | 1-12 months | 45 RSV-AB; 27 healthy controls | Clinical severity score; need for oxygen supplement |
| MIP-1α | *Bermejo-Martin et al, 2007*[27] | (-) | < 24 months | 22 RSV LRTI; 22 healthy controls | Wood′s Clinical Asthma Score (M-WCAS) scoring system |
| *Moreno-Solis et al, 2015*[55] | (na) | 1-12 months | 45 RSV-AB; 27 healthy controls | Clinical severity score; Need for oxygen supplement |
| PDGF-ββ | *Bermejo-Martin et al, 2007*[27] | (+) | < 24 months | 22 RSV LRTI; 22 healthy controls | Length of hospital stay, Oxygen saturation (positive correlation with RSV severity)  |
| sCD25 | *Fernández et al, 2005*[47] | (+) | < 12 months | 196 infants with RSV infection | Hypoxia (oxygen saturation < 95%) versus non-hypoxia |
| TNF-α | *Geevarghese et al, 2014*[56] | (+) | 1 month - 5 years | 29 infants with RSV infection | Length of hospitalization |
| *Mella et al, 2013*[52] | (na)\* | < 24 months | 66 RSV LRTI; 14 healthy controls | Admission to PICU (severe); floor patients (mild-moderate). Severity is defined by a scoring system (length of hospitalization; the need for supplemental oxygen; duration of supplemental oxygen)\*(-) TNF-α production capacity is associated with length of stay and RSV severity score |
| *Brandenburg et al, 2000*[48] | (na) | < 6 months | 111 hospitalised infants (50 confirmed severe, 45 mild RSV infection) | Severe infection: pCO2 > 6,6kPa, oxygen saturation < 90% or need for mechanical ventilation; mild infection: other hospitalised infants |
| *Larrañaga et al, 2009*[49] | (na) | < 6 months | 75 infants with RSV infection (37 severe, 38 moderate); 24 healthy controls | Clinical severity score: Hospitalisation; need for supplemental oxygen; no maximal FIO2 (%) |
| *Hornsleth et al, 1998*[35] | (na) | < 4 years | 105 RSV LRTI | Clinical severity score: Respiratory rate; alveolar or peri bronchial infiltration; respiratory support; duration of hospitalisation |
| *Vieira et al, 2010[47]* | (na) | < 3 months | 30 RSV LRTI | Clinical severity score (respiratory rate; wheezing; oxygen saturation; accessory muscle recruitment); duration of oxygen therapy; duration of mechanical ventilation; duration of hospitalisation |
| *Moreno-Solis et al, 2015*[55] | (na) | 1-12 months | 45 RSV-AB; 27 healthy controls | Clinical severity score; need for supplemental oxygen |
| VEGF | *Bermejo-Martin et al, 2007*[27] | (+) | < 24 months | 22 RSV LRTI; 22 control infants | Oxygen saturation (positive correlation with RSV severity) |

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