Chapter 3

Dynamics of viral load and symptoms in infants hospitalised with respiratory syncytial virus or rhinovirus lower respiratory tract infection

Janneke C van Leeuwen
Ron MGR Hendrix
Mirjam R Meilink
Eric J Duiverman
Boony J Thio

Work in progress
ABSTRACT

Background
Viral lower respiratory tract infection (VLRTI) in infancy is often caused by the respiratory syncytial virus (RSV) or rhinovirus (RV). Several studies have described a decreasing RSV viral load during disease and a relation between RSV viral load and disease severity. Little is known about the dynamics of viral load during a RV VLRTI.

Objective
To compare the dynamics of the viral load in relation to clinical features in infants hospitalised for a RSV or RV VLRTI.

Methods
In this prospective study we recruited infants up till age 2, presenting with a VLRTI requiring hospitalisation. Upon hospital admission, pathogens and viral load were determined. Clinical symptoms were documented with a validated questionnaire. At hospital discharge and outpatient check-up, viral load and symptom scores were determined again.

Results
103 Infants were included, 38 completed the full study procedure. In RSV VLRTI, viral load significantly decreased during hospitalisation. In RV VLRTI, viral load remained stable during the study period. There was a correlation between RSV viral load at hospital admission and duration of hospitalisation ($r=0.49$, $p=0.001$). In RV VLRTI, there was no correlation between viral load and disease severity.

Conclusion
RSV viral load at hospital admission is related to duration of hospitalisation and decreases during hospitalisation. In contrast, RV viral load remains stable during disease and does not predict duration of hospitalisation. The different dynamics of RSV and RV viral load suggest a different pathophysiological mechanism, which might be important in explaining the relation between VLRTI and airway disease in later life.
INTRODUCTION

Viral lower respiratory tract infection (VLRTI) in infancy is a common cause of hospitalisation with a high morbidity\(^1\). Respiratory syncytial virus (RSV) and rhinovirus (RV) are the predominant viruses, associated with severe VLRTI leading to hospitalisation in infancy\(^2-7\).

With the availability of Real Time -Polymerase Chain Reaction (RT-PCR), viral pathogens can be rapidly identified in specimens from nasopharyngeal washings\(^8\). Because of the aggravating nature of this washing, a nasal swab has been developed, which is less invasive and proved to be as sensitive as a nasopharyngeal washing in identifying a viral pathogen\(^9-11\). The number of amplification cycles needed for a positive PCR test (cycle threshold, CT-value) is inversely linearly related to the viral load of a pathogen\(^12\). Several studies have investigated the relation between RSV viral load and disease severity in infants hospitalised with VLRTI, but the results are inconclusive\(^2,13-16\). The relation between viral load and disease severity in infants hospitalised with RV VLRTI is unclear\(^7,14\). The dynamics of the viral load during VLRTIs was investigated in a small number of studies that mainly focused on RSV\(^14,16,17\). The purpose of this prospective study is to compare the dynamics of the viral load in relation to clinical features in infants hospitalised for a RSV or RV VLRTI.

METHODS

Subjects

This prospective study was conducted from August 2010 till April 2011. We recruited infants up till age 2, who were hospitalised with a clinical diagnosis of VLRTI at Medisch Spectrum Twente, Enschede, the Netherlands (a large community and teaching hospital). An experienced clinician diagnosed VLRTI on clinical grounds with symptoms of rhinorrhea, cough, dyspnea or wheezing with or without fever. Infants with pulmonary or cardiac co-morbidity were excluded from participation. Infants with a history of prematurity could be included, if they had no pulmonary or cardiac co-morbidity. The study was conducted after approval of the local Ethics committee. All parents received written patient information and signed an informed consent form before study entry.

Study design

Upon hospital admission, nasopharyngeal specimens from all included infants were obtained using a nasal flocked swab (FLOQSwabs by Copan, CE0123). In order to validate follow-up sampling with nasal swabs, a nasopharyngeal washing was taken at hospital admission as well. The washing was taken after the nasal swab, by flushing the nasophar-
ynx with 2 ml of 0.9% sodium chloride solution. An experienced clinician documented clinical symptoms, using a structured symptom score questionnaire according to Gern et al., which is used in several other studies investigating VLRTI in infants. Scores 0-4 indicated mild infection, 5-10 indicated moderate infection and scores >11 indicated severe infection. During hospital admission, infants were treated following current guidelines, which mainly exist of supplemental oxygen administration and securing of hydration. At hospital discharge and at outpatient check-up, two to three weeks after discharge, nasal swabs and symptom scores were performed again.

**RT-PCR and viral load**

Nasopharyngeal specimens were directly transported to the laboratory and immediately processed or stored at -80°C until processing. RT-PCR, performed on both specimens, could detect Influenza virus type A/B, Parainfluenza 2/4 virus (PIV 2/4), PIV 1, PIV 3, Adenovirus, Respiratory Syncytial A/B virus, Rhinovirus, Human Metapneumovirus, Chlamydia pneumoniae, Mycoplasma pneumoniae and Enterovirus. Separate assays were performed for each target. In all specimens, the viral load was semi-quantitative determined by the number of amplification cycles needed for a positive PCR test (cycle threshold, CT). CT-values of 40 and lower demonstrating a characteristic amplification plot, were considered positive. Quality of taken samples was randomly checked through detection of the amount of cells by beta-globulin reaction.

**Statistical analysis**

Results were expressed as mean values ± standard deviation (SD) for normally distributed data, as median (minimum-maximum) for not normally distributed data or as numbers with corresponding percentages if nominal or ordinal.

Continuous variables were tested for normality with a Shapiro-Wilk or Kolmogorov-Smirnov test as appropriate. Differences in clinical characteristics between children with positive and negative laboratory findings were determined by using Chi-Square test, Fischers exact test, Mann-Witney U test and independent samples t-test (if normally distributed). Dynamics of viral load were determined by using a paired samples t-tests or Wilcoxon-signed rank sum test as appropriate. Correlations were calculated using Spearman correlation. A 2 sided value of p<0.05 was considered statistically significant. All analyses were performed with the Statistical Package for the Social Sciences (SPSS®) for Windows® version 15 (IBM, Chicago, IL, USA).
RESULTS

Study population
103 Infants with a VLRTI requiring hospitalisation were included during the study period, of which 38 completed the full study procedure. In 38 infants, samples were only taken at hospital admission. In the remaining 27 infants study procedure was partially completed. Table 1 shows the baseline characteristics of the study population.

RT-PCR identified one or more pathogens in 98 infants (95.1%). Twenty-nine infants (28.2%) were infected with 2 or 3 pathogens. RSV and RV were the predominant pathogens identified, respectively in 62 (60.2%) and 46 infants (44.7%), co-infections included.

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics.</th>
<th>Included children (N=103)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)</td>
<td>6.1 (0.3;22.6)</td>
</tr>
<tr>
<td>Male</td>
<td>62 (60.2)</td>
</tr>
<tr>
<td>History of prematurity</td>
<td>13 (12.6)</td>
</tr>
<tr>
<td>Symptom scores:</td>
<td></td>
</tr>
<tr>
<td>Admission</td>
<td>15 (1;24)</td>
</tr>
<tr>
<td>Discharge</td>
<td>2 (0;14)</td>
</tr>
<tr>
<td>Outpatient check-up</td>
<td>1 (0;19)</td>
</tr>
<tr>
<td>Duration of hospitalisation (days)</td>
<td>3 (0;9)*</td>
</tr>
</tbody>
</table>

Results expressed as median (minimum;maximum) or as numbers (percentages). *1 infant was admitted <24 hours

Nasal swab versus nasopharyngeal washing
In order to validate follow-up sampling with nasal swabs, a nasopharyngeal washing was taken at hospital admission as well in 96 out of 103 infants. Analysis of these paired specimens showed that nasal swabs are very accurate in detecting RSV (positive predicting value 100%, negative predicting value 95%, sensitivity 97% and specificity 100%) and accurate in detecting RV (positive predicting value 94%, negative predicting value 88.5%, sensitivity 82.5%, specificity 96.4%). CT-values determined with the nasal swab were significantly higher than CT-values determined with nasopharyngeal washings (in RSV; median CT-value washing 23.5 (18;38), swab 26 (19;40), p<0.001, in RV; median CT-value washing 28 (18;35), swab 31 (21;39), p<0.001).

CT-value and clinical features
The relation between viral load and clinical features was investigated in the two most prevalent pathogens, respectively RSV (N=43) and RV (N=23) (co-infections not included). Besides duration of hospitalisation (median 4 days (range 1;8) in RSV VLRTI, versus
2 days (range 1;5) in RV VLRTI, p<0.01), there were no significant differences in baseline characteristics between both groups. CT-value at hospital admission, determined with nasal swabs, was significantly higher in RV infected infants compared to RSV infected infants; median CT-values respectively 29 (21;36) versus 25 (20;40), p=0.02.

There was a correlation between viral load at admission and duration of hospitalisation in infants with a RSV VLRTI (r=0.49, p=0.001). This correlation was only found in viral load determined with nasal swabs. There was no relation between RSV viral load at admission and symptom score. Four infants (9%) with RSV VLRTI had a history of prematurity (mean gestational age: 35+4/7 weeks). There were no differences in length of hospitalisation (p=0.82) or symptom score (p=0.52) between infants with or without a history of prematurity.

In RV VLRTI, there was no correlation between viral load at admission and duration of hospitalisation or symptom score.

**Dynamics of viral pathogens**

At hospital discharge the same pathogen was identified by RT-PCR as at admission in 75.9% of the infants (44 out of 58). At outpatient check-up 2-3 weeks after discharge 17.8% (8 out of 45 infants) still had a positive RT-PCR for the same pathogen. RSV persisted in 88% of the RSV infected infants at hospital discharge and in 10% at outpatient check-up. RV persisted in 68% of the RV infected infants at hospital discharge and in 20% at outpatient check-up.

During hospital admission 6.9% of the infants (4 out of 58) was colonised with another pathogen (2x PIV, RSV and RV).

There was no difference in duration of hospitalisation (median 3 days) or symptom scores at hospital discharge (median 2) and outpatient check-up (median 1) between infants with or without persistent positive RT-PCR, as analysed in all infants.

**Dynamics of viral load**

The dynamics of the viral load in RSV and RV VLRTI is shown in figure 1. Because of the limited data of viral load in single infections, viral load of RSV and RV in co-infections was also analysed. In RSVVLRTI (N=59), CT-value significantly increases during hospitalisation (median CT-value admission; 26[19;40], median CT-value discharge; 34[24;40], p<0.001), indicating a decrease in viral load. In RV VLRTI (N=36), viral load seems to be constant (31.5[21;39], 31[27;35], 30.5[29;37]) with no significant changes in viral load between hospital admission and discharge or follow-up; p=0.91 and p=0.28). One infant with a RSV VLRTI was treated with systemic steroids, which was already started before hospital admission. None of the infants had a history of palivizumab immunisation. 13 Out of 36 (36%) analysed children with RV VLRTI and 27 out of 59 (46%) analysed children with RSV
VLRTI were treated with antibiotics during hospitalisation. The dynamics of the viral load did not differ between infants with or without antibiotic treatment.

**DISCUSSION**

Our results show that in infants with a RSV VLRTI, viral load at hospital admission is related to duration of hospitalisation and viral load significantly decreases during hospitalisation. Thus, RSV viral load at hospital admission may be used as a prognostic measure to predict the clinical course of VLRTI. In contrast, viral load in RV VLRTI remains stable during and after hospitalisation, and viral load at hospital admission does not predict duration of hospitalisation.

To our knowledge this study is the first investigating the dynamics of viral load with RT-PCR, during and after hospitalisation in infants with RSV or RV VLRTI. Few studies have investigated the dynamics of viral load in RSV VLRTI and also observed a decreasing RSV viral load during hospitalisation. In addition, Saleeby et al. and Martin et al. described the predictive value of viral load for clinical severity of RSV infections; increased viral load was associated with prolonged hospitalisation. The dynamics of RV viral load during hospitalisation was only investigated in a large study conducted by Franz et al. This study was performed in children under 16 years of age, hospitalised for a lower respiratory tract infection. This population differs from our study, where we investigated infants under 2 years of age. Franz et al. determined viral load with RT-PCR at hospital admission and at the 3rd or 4th day of hospitalisation. In accordance to our results, they found no significant change in RV viral load during hospitalisation.
In our study, RT-PCR identified a pathogen in 95.1% of the infants, which is high compared to other studies\textsuperscript{3,4,7,14}. Corresponding to other studies, RSV (60.2%) and RV (44.7%) were the predominant pathogens causing VLRTI in infancy\textsuperscript{2,6,7}. A co-infection was detected in 28.2% of the hospitalised infants. Remarkably, 6.9% of the hospitalised infants was colonised with another viral pathogen during hospital stay. Although the source of this infection is hard to trace, this colonisation is probably due to transmission via parents, nurses or clinicians, despite taken precautionary measures to prevent intramural transmission of pathogens.

Comparing the clinical characteristics and viral loads of infants with a RSV VLRTI and RV VLRTI, a few points need to be mentioned. First, median CT-value at hospital admission is higher in RV VLRTI compared to RSV VLRTI, which is consistent with other research\textsuperscript{7}. The meaning of this difference is unclear, but suggests a different pathophysiological mechanism in RSV and RV infections. Secondly, duration of hospital stay was longer in infants with a RSV VLRTI compared to RV VLRTI. These results are in contrast with several former studies, describing a similar clinical pattern in RSV and RV VLRTI\textsuperscript{3,5,7}. Symptom scores at hospital admission did not differ between RSV and RV VLRTI. Possibly, the above mentioned differences are due to the seasonal variance in virulence of viral pathogens.

Our finding that a nasal swab, as compared to a nasopharyngeal washing, is a sensitive (respectively 97% and 82.5%) manner to identify RSV and RV in infants hospitalised for a VLRTI, has already been described by others\textsuperscript{9-11}. In the 12 cases that nasal swabs were false negative, the identified pathogens with nasopharyngeal washings had a relatively high (34.1) mean CT-value, which indicates low viral load.

A potential limitation of our study is the amount of missing data. It was not possible to perform a correct follow-up for all infants, as was the case in similar studies\textsuperscript{17}. This is probably due to the demanding work at the children’s department in the winter season and the large number of health workers that is involved in the care of a hospitalised infant. We tried to optimise follow-up by regularly informing all involved health workers and we believe that the obtained data provide enough evidence for the results described. Another remark must be made about the small number of infants with a RV VLRTI compared to RSV VLRTI. This, in combination with the relatively short hospital stay of infants with a RV VLRTI, could influence the analysis of the relation between RV viral load and length of hospitalisation. On the other hand, we consider that such a relation is unlikely, since RV viral load remains stable during disease. Moreover, our results are consistent with previous studies, showing no relation between RV viral load and disease severity\textsuperscript{7,12}.

The findings of this study are of clinical importance, as they indicate that RSV viral load has predictive value for the clinical course of VLRTI. The different dynamics of RV and RSV viral load during disease might be due to a different pathophysiological mechanism. We hypothesise that in RSV VLRTI, viral replication induces direct cytopathic effects, explain-
ing the relation between viral load and disease severity. In contrast, disease severity in RV VLRTI might be more related to the severity of the immunopathologic response to the viral infection, explaining the lack of a relation between viral load and disease severity. Genetic predisposition to an immunopathologic response to RV infection may contribute to later airway disease\textsuperscript{16}. Further research is necessary to investigate this and to define thresholds for RSV viral load at which advanced care is anticipated.

In conclusion, RSV viral load, determined by CT-value, decreases during hospitalisation. In combination with the finding that viral load is related to length of hospital stay in RSV VLRTI, viral load could be used as a prognostic and monitoring measure to predict the clinical course in RSV VLRTI. In contrast, viral load in RV VLRTI remains stable during and after hospitalisation and does not predict duration of hospitalisation. The different dynamics of RV and RSV viral load in VLRTI suggest a different pathophysiological mechanism. The persistence of the viral load in RV infections may induce an immunopathologic process, leading to chronic inflammatory airway disease. This could explain the strong relation between RV VLRTI and airway disease in later life\textsuperscript{20,21}. 


REFERENCES


