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Correlation of viral load as determined by real-time RT-PCR and clinical characteristics of respiratory syncytial virus lower respiratory tract infections in early infancy

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Abstract

Background: In infants hospitalized for a lower respiratory tract infection (RTI) caused by respiratory syncytial virus (RSV), the correlation between viral load (VL) and patient clinical characteristics remains to be defined.
Objectives: To define this correlation.
Study design: prospective study of 47 infants admitted to hospital in the period November 2006–May 2007 with a diagnosis of lower RTI. Nasopharyngeal aspirates (NPAs) were taken at admission, discharge, and at post-discharge control visits. VL was quantified by real-time RT-PCR for RSV subgroups A and B.
Results: Patients with bronchiolitis were compared with young patients with lower RTI other than bronchiolitis. Patients with bronchiolitis had a significantly lower age than patients with other syndromes, and a significantly longer duration of symptoms. Duration of hospitalization was not different in the two groups of patients, and was not related to RSV subgroup or viral coinfection. A sustained decrease in VL was observed in the general patient population between admission, discharge and post-discharge follow-up visits.
Conclusions: (i) patients with bronchiolitis were significantly younger than patients with other lower RTIs; (ii) symptom duration was significantly longer in patients with bronchiolitis; (iii) RSV VL significantly decreased between admission and discharge.

Keywords: Respiratory syncytial virus; Lower respiratory tract infection; Real-time RT-PCR; Nasopharyngeal aspirate; Viral load in NPA

1. Introduction

Respiratory syncytial virus (RSV) is the major viral pathogen of the respiratory tract in early infancy and, due to its frequent involvement of the lower respiratory tract, is recognized as the single most important cause of infant hospitalization (Boyce et al., 2000; Leader and Kohlhase, 2002). Following the use of quantitative reverse transcription (RT)-PCR (Campanini et al., 2007; Falsey et al., 2003), in recent years, real-time RT-PCR has become the method of choice for quantification of RSV load in nasopharyngeal aspirates (NPAs) taken during acute respiratory tract infections (RTI) (Perkins et al., 2005).

In this study, by using a real-time RT-PCR approach, quantification of RSV load in NPAs from 47 infants admitted to hospital with a lower RTI was investigated with respect to: (i) duration of hospitalization; (ii) drop in viral load during patient follow-up and symptom resolution; (iii) RSV subgroups (A and B); (iv) patient subdivision into two groups: one, including patients with bronchiolitis ($n = 18$), and the

Abbreviations: RSV, respiratory syncytial virus; RTI, respiratory tract infection; RT-PCR, reverse transcription-PCR; NPA, nasopharyngeal aspirate.

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Table 1
Comparison of young patients grouped by clinical syndrome, RSV subgroup, and single RSV infection or coinfection

Clinical parameter	Hospitalization duration (days)	*p*
Patients with bronchiolitis ($n=18$) *vs.* patients with other lower ARTI ($n=29$)	8 (4–11) *vs.* 7 (2–16)	ns
Patients with RSV-A ($n=25$) *vs.* patients with RSV-B infection ($n=22$)	8 (3–11) *vs.* 8 (2–16)	ns
Patients with coinfection ($n=15$) *vs.* patients with single RSV infection ($n=32$)	9 (6–11) *vs.* 8 (2–16)	ns

other one including patients with lower RTI other than bronchiolitis ($n=27$). Viral load in the two subgroups was analysed with respect to patient age, duration of symptoms and RSV subgroup.

2. Patients and methods

On the whole, 47 infants with a median age of 5 (1–27) months, admitted to the hospital with a diagnosis of lower RTI in the period November 2006–May 2007, were examined in NPA by real-time RT-PCR for RSV RNA quantification, according to two reported protocols for RSV (subgroups A and B) quantification (Perkins et al., 2005). The intra-assay coefficient of variation (CV) for real-time RT-PCR was 8.53% for RSV-A and 9.39% for RSV-B, while the inter-assay CV was 35.8% for RSV-A, and 31.5% for RSV-B. Besides the 18 infants with bronchiolitis, of the 29 young patients with lower RTI other than bronchiolitis, 20 had pneumonia, 6 bronchitis, and 3 wheezing. NPAs were taken according to a standardized procedure only at admission to hospital from 15 infants, both at admission and discharge from 22 infants, and, besides admission and discharge, also at one or more control medical visits after discharge, anyway within 30 days after admission. The median time interval between NPAs taken at admission and discharge was 7 (2–15) days, while the median interval between admission and the first medical visit after discharge was 21 (17–29) days.

3. Results

The duration of hospitalization was found to be comparable ($p=$ ns) in infants with bronchiolitis *versus* infants with a lower RTI other than bronchiolitis [8 (4–11) *vs.* 7 (2–16) days]. In addition, hospitalization was not different ($p=$ ns) in infants with RSV-A infection *versus* infants with RSV-B infection [8 (3–11) *vs.* 8 (2–16) days], as well as in young patients with coinfection *versus* patients with a single RSV infection [9 (6–11) *vs.* 8 (2–16) days] (Table 1). Drop in viral load was significant between admission and discharge and between discharge and subsequent controls (Fig. 1A).

A significant correlation was found between viral load reduction in NPAs and the time interval between NPAs taken at admission and discharge (Fig. 1B). This indicates that, since patients were discharged from the hospital concomitantly with resolution or amelioration of clinical symptoms, there was a direct relationship between level of viral load in NPA and clinical symptoms. This relationship was not found between viral load reduction in NPA and the time interval between admission to hospital and control visits after discharge. This was due to the fact that in 7/10 infants tested in NPA after discharge, RSV persisted in respiratory secretions, and was shed at a low level for at least some weeks after patient discharge. On the other hand, no difference in viral load at admission was found between infants with RSV-A ($n=25$) *versus* infants with RSV-B ($n=22$) lower RTIs.

When the two groups of patients with bronchiolitis and non-bronchiolitis lower RTI were compared, it was found that (i) patients with bronchiolitis ($n=18$) were significantly ($p=0.025$) younger [median age 3.5 (1–10) months] than

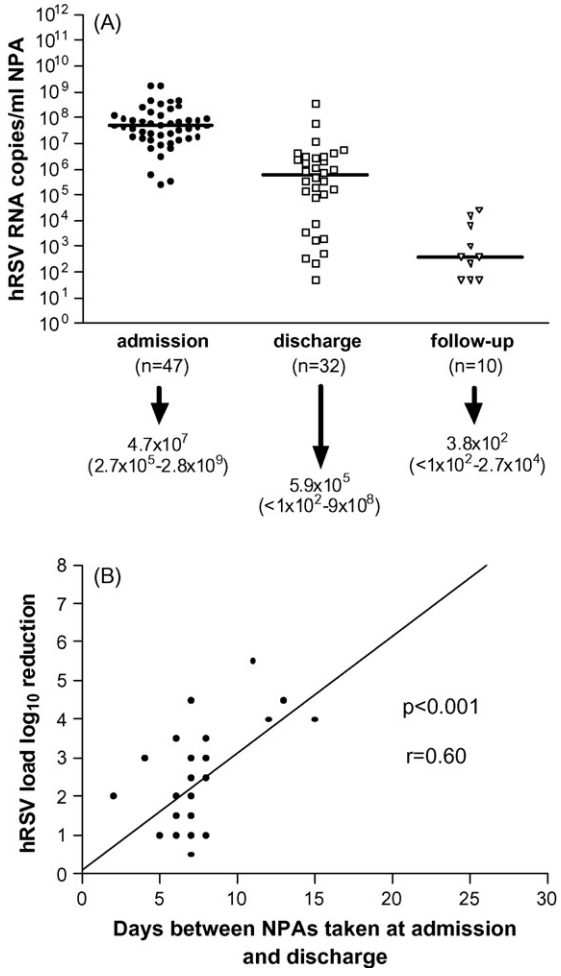

Fig. 1. (A) Median drop in viral load in sequential NPAs taken at admission, discharge and follow-up. (B) Correlation between viral load reduction and interval between NPAs taken at admission and discharge.