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Does respiratory syncytial virus subtype influences the severity of acute bronchiolitis in hospitalized infants?

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Summary Respiratory syncytial virus (RSV) subtypes A and B are present either simultaneously or alternate during yearly epidemics. It is still not clear whether clinical severity of acute bronchiolitis differs between the two subtypes.

Reverse transcription polymerase chain reaction was used to subtype RSV in previously healthy infants hospitalized with RSV bronchiolitis during a winter epidemic. A severity index based on heart rate, respiratory rate, wheezing, difficulty in feeding and oxygen saturation was calculated upon admission.

Infants infected with RSV subtype-A were found to have a significantly higher (more severe) clinical score than those infected with RSV-B. There was no statistically significant difference in duration of hospitalization or need of intensive care. Boys and infants younger than 3 months of age were also more severely affected than girls or older infants, respectively.

These results support the notion that RSV-A-induced bronchiolitis is more severe than RSV-B-induced one, in agreement with the majority of previously published studies.

Introduction

Bronchiolitis is an acute respiratory illness of children in the first 2 years of life mostly associated with respiratory syncytial virus (RSV).[1,2] Two RSV subtypes, A and B have been identified[3] with antigenic differences sufficiently extensive to affect susceptibility to infection or disease.[4] However, direct comparisons between subtypes in terms of disease severity have produced controversial results. RSV-A[5–8] or RSV-B[9] were found to result in more severe infection, however, an equal number of investigations have failed to confirm any difference.[10–12] These discrepancies could be attributed to differences in study design, which in most cases has been retrospective, disease definition, inclusion criteria, outcome measures or subtyping methodology. Furthermore, it is possible that strain virulence may be variable between epidemics. As more therapeutic and prophylactic modalities against RSV are becoming available, possible differences in disease severity between subtypes may in the future have an effect on treatment strategies. We hypothesized that there

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may be differences in the severity of bronchiolitis caused by different RSV subtypes and aimed to investigate prospectively any such differences in infants hospitalized with the disease.

Material and methods

Study subjects

Eighty-one infants (45 boys) with mean age 3.5 ± 0.3 months (range 0.5–12) hospitalized with a clinical diagnosis of bronchiolitis and positive RSV immunofluorescence test were included in the study. Bronchiolitis was defined as an acute infection of the lower airway characterized by increased respiratory effort (>50 respirations/min and/or use of accessory respiratory muscles), and expiratory wheezing and/or crackles. A detailed questionnaire and a nasopharyngeal aspirate (NPA) were obtained upon admission. Disease severity was assessed based on heart rate, respiratory rate, wheezing, evidence of cyanosis or difficulty of feeding, and oxygen saturation as previously described.[13] Infants with underlying chronic diseases or recurrent (>2) wheezing episodes were excluded. Informed consent was obtained from parents. Infants were managed and discharged according to routine clinical practices.

RSV typing

RSV-A and -B subtypes were characterized by a multiplex nested reverse transcription-PCR (RT-PCR), according to a published protocol.[14] Primers were obtained from MWC-Biotech, Munich, Germany. Amplicons were visualized by ethidium bromide staining after electrophoresis on a 1.5% agarose gel (Gibco). Amplicon size (RSV-A = 334, RSV-B = 183) was used to differentiate between the subtypes. Reference viral strains were included as positive controls; several negative controls were also included in each run.

Statistical analysis

Continuous variables were analyzed with one-way analysis of variance (ANOVA) and Student's *t*-test; chi-square was performed for ordinal or categorical data. Continuous data are expressed as mean ± standard error of mean. $P<0.05$ was considered significant.

Results

Forty-eight samples were positive for either RSV-A or RSV-B by PCR (59%). Sub-grouped cases had more frequently a positive family history of atopy (37.0% vs. 15.6%, $P=0.039$) and a marginally higher severity index (8.79 ± 0.2 vs. 8.15 ± 0.3, $P=0.075$, *t*-test), with no other differences in demographic or disease characteristics. Of the sub-grouped samples 23 (48%) were RSV-A and 25 (52%) RSV-B. Table 1 summarizes the data in the three groups (RSV-A, RSV-B, non-subtyped). Age, sex, birth weight, day of disease at admission, duration of hospitalization, pre or postnatal exposure to tobacco smoke, family history of atopy, presence of fever, leukocyte count and differential, ESR, CRP and IgE had no significant differences in respect to subtype (data not shown). Moreover, there was no difference in the percentage of infants with a consolidation in their chest radiograph (RSV-A 27%, RSV-B 19%, non-typed 22%, P = non-significant). Severity index was significantly higher in patients where RSV-A was identified (RSV-A: 9.3 ± 0.4, RSV-B: 8.4 ± 0.3, non-subtyped: 8.2 ± 0.3, $P=0.031$,

Table 1 Demographic and clinical characteristics of patients infected with RSV-subtype A, RSV-subtype B or non-typed cases. ns = non-significant.

Variable	RSV-A $n=23$	RSV-B $n=25$	Non-typed $n=33$	P
Age	3.7 ± 0.6	3.2 ± 0.5	3.5 ± 0.5	ns
Sex (male)	13/23, 57%	11/25, 44%	21/33, 64%	ns
Birth weight	3104 ± 104	3200 ± 90	3087 ± 95	ns
Day of disease at admission	3.8 ± 0.4	3.2 ± 0.4	3.1 ± 0.3	ns
Duration of hospitalization (days)	5.4 ± 0.6	6.2 ± 1.3	5.1 ± 0.5	ns
No exposure to tobacco smoke	5/19, 26%	10/24, 42%	10/33, 30%	ns
Family history of atopy	7/22, 32%	10/24, 42%	5/32, 16%	ns
Fever (⩾38°C)	11/22, 50%	9/25, 36%	15/33, 46%	ns
Severity index*	9.3 ± 0.4	8.4 ± 0.3	8.2 ± 0.3	0.031

*Based on cardiac rate, respiratory rate, presence of wheezing, skin colour and O_2 saturation.[13]

Table 2 Average values of individual clinical parameters used to calculate the severity index, by RSV subtype. Each parameter was scored with a 1–3 scale of increasing severity.[13] The differences are not statistically significant.

Variable	RSV-A	RSV-B
Heart rate	1.74 ± 0.13	1.60 ± 0.12
Respiratory rate	2.30 ± 0.12	2.04 ± 0.14
Wheezing	1.09 ± 0.09	1.04 ± 0.04
Skin color/feeding	1.48 ± 0.15	1.21 ± 0.08
Sat O_2	2.65 ± 0.12	2.63 ± 0.10

RSV-A vs. RSV-B, $P = 0.049$). All clinical parameters were more affected in infants with RSV-A infection, however these differences were not statistically significant (Table 2).

All infants received oxygen supplementation until 1 day before discharge. Antibiotics were given to 6/23 (26%) of the RSV-A group and 6/25 (24%) of the RSV-B; systemic steroids to 7/23 (30%) of the RSV-A group and 4/25 (16%) of the RSV-B (not statistically significant). One infant from each RSV subtype was admitted to the intensive care unit.

Boys had more severe disease than girls (8.96 ± 0.22 vs. 8.00 ± 0.27, $P = 0.007$). Infants <3 months ($n = 42$) had more severe disease than older ones (8.86 ± 0.24 vs. 8.18 ± 0.26, $P = 0.058$). Prematurity, low birth weight, family history of atopy, fever $\geqslant 38^{\circ}C$, exposure to tobacco smoke currently or during pregnancy, and number of siblings were not different between the groups.

Discussion

In this study, using a composite index that takes into account several clinical parameters,[13] it was shown that the severity score of infants infected with RSV-A was higher than the one of infants infected with RSV-B, or non-subtyped RSV. The difference was small but statistically significant. Length of hospitalization was not significantly different between the groups, possibly because this parameter is based upon clinical decisions that cannot be controlled. Young age and male sex affected disease severity, consistently with other studies.[12]

An unexpected weakness of this study was the considerable number of samples that could not be subtyped by PCR. Lower viral load and/or the presence of PCR inhibitors in the samples may account for this finding. The method used may be slightly less sensitive for RSV-B,[14] however, even in this case, the reported increased severity of RSV-A would be strengthened. In a recent study, Hornsleth and colleagues used PCR for subtyping RSV; however, they performed the PCR reaction on viral isolates derived from culture where a very high viral concentration is present, in contrast to nasal aspirate samples.[9]

The discrepancies in the results of different studies looking into RSV subtype and bronchiolitis severity need to be further elucidated.[4,5,7,8,9,11] Most of the studies have found RSV-A as the most severe pathogen;[5–8] however in some cases, this effect was lost when confounders were taken into account in logistic regression models.[10] This could mean that the correlation either does not exist, or that the power of the study was not enough to confirm it. In this respect, a meta-analysis of the various studies could be useful; however, inclusion criteria, disease definitions, outcome measures and virus identification methodologies differ widely, thus making such an approach difficult.

In conclusion, RSV subtype A infection resulted in more severe bronchiolitis in this cohort of hospitalized infants, as in the majority of published studies. Difference in severity was small and should be interpreted with caution. However, it is possible that no single study may be able to account for all variation between years, locations, immune status of the community and virulence. This indicates the need for studies with well-defined inclusion criteria and outcome measures in order to allow for successful meta-analyses.

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