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Original Article

Viral Coinfection in Childhood Respiratory Tract Infections☆

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ABSTRACT

Introduction: The introduction of molecular techniques has enabled better understanding of the etiology of respiratory tract infections in children. The objective of the study was to analyze viral coinfection and its relationship to clinical severity.
Methods: Hospitalized pediatric patients with a clinical diagnosis of respiratory infection were studied during the period between 2009 and 2010. Clinical and epidemiological data, duration of hospitalization, need for oxygen therapy, bacterial coinfection and need for mechanical ventilation were collected. Etiology was studied by multiplex PCR and low-density microarrays for 19 viruses.
Results: A total of 385 patients were positive, 44.94% under 12 months. The most frequently detected viruses were RSV-B: 139, rhinovirus: 114, RSV-A: 111, influenza A H1N1-2009: 93 and bocavirus: 77. Coinfection was detected in 61.81%, 36.36% with two viruses, 16.10% and 9.35% with three to four or more. Coinfection was higher in 2009 with 69.79 vs 53.88% in 2010. Rhinovirus/RSV-B on 10 times and RSV-A/RSV-B on five times were the most detected coinfections. Hospitalization decreased with greater number of viruses ($P<.001$). Oxygen therapy was required by 26.75% (one virus was detected in 55.34% of cases). A larger number of viruses resulted in less need for oxygen ($P<.001$). Ten cases required mechanical ventilation, four patients with bacterial coinfection and five with viral coinfection ($P=.69$).
Conclusions: An inverse relationship was found between the number of viruses detected in nasopharyngeal aspirate, the need for oxygen therapy and hospitalization days. More epidemiological studies and improved quantitative detection techniques are needed to define the role of viral coinfections in respiratory disease and its correlation with the clinical severity.

Coinfección vírica en las infecciones respiratorias infantiles

Palabras clave:
Coinfección
Infecciones de vías respiratorias
Virus respiratorio sincitial

RESUMEN

Introducción: Las técnicas moleculares han permitido un mejor conocimiento de la etiología de las infecciones respiratorias infantiles. El objetivo del estudio fue analizar la coinfección viral y su relación con la gravedad clínica.
Métodos: Se estudió a pacientes pediátricos hospitalizados con diagnóstico clínico de infección respiratoria durante el periodo comprendido entre 2009 y 2010. Se recogieron datos clínicos, epidemiológicos, duración de la hospitalización, necesidad de oxigenoterapia, coinfección bacteriana y necesidad de ventilación mecánica. Etiología estudiada con técnica PCR múltiple y microarrays de baja densidad para 19 virus.
Resultados: Un total de 385 pacientes presentaron resultados positivos, 44,94% menores de 12 meses. Los virus más detectados fueron: VRS-B: 139, rhinovirus: 114, VRS-A: 111, influenza A H1N1-2009: 93 y bocavirus: 77. Se detectó coinfección en el 61,81%, un 36,36% con 2 virus, 16,10% con 3 y 9,35% con 4 o más. La coinfección fue superior en 2009 con 69,79 frente 53,88% en 2010. Rhinovirus/VRS-B en 10 ocasiones y VRS-A/VRS-B en 5 fueron las coinfecciones más detectadas. Menor hospitalización a mayor número de virus detectados ($p < 0,001$). Necesitaron oxigenoterapia el 26,75% (en 55,34% se aisló un virus), objetivando a mayor número de virus menor necesidad de oxígeno ($p < 0,001$). Precisaron ventilación mecánica 9 casos, 4 de ellos con coinfección bacteriana y 5 con coinfección vírica ($p = 0,69$).

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Conclusiones: Objetivamos una relación inversamente proporcional entre número de virus detectados en aspirado nasofaríngeo, necesidad de oxigenoterapia y días de hospitalización. Se necesitan más estudios epidemiológicos y mejoría en las técnicas de detección cuantitativa para definir el papel de las coinfecciones víricas en la enfermedad respiratoria y su correlación con la gravedad clínica.

Introduction

Respiratory viral infection is a major source of morbidity and mortality in childhood. Just over one-third of preschool-aged children develop lower respiratory infections during their first year of life. Between 1% of 2% of these patients require admission to hospital.[1,2]

The etiological agent is not always identified in these patients. Children with respiratory infections are generally treated as outpatients and the etiology of their disease is not investigated. If they are hospitalized, the commonly employed evaluation techniques are often not sufficiently sensitive.[3]

Experience and understanding of the role of viral coinfections in respiratory infections has grown in recent years, thanks to the introduction of molecular techniques.[4–6] At present, the clinical data available on coinfection, in terms of both the number of viruses involved and the severity of the condition, are variable and even, at times, contradictory. These discrepancies may be due to factors that affect the various etiological agents, such as geographical region and methods of detection employed.

The aim of this study was to analyze the viral etiology of respiratory infections and the real clinical implications of coinfection, and its possible relationship to clinical severity in a population of hospitalized patients in a general hospital located in Barcelona with a secondary-level pediatric department. Etiology was determined using multiplex polymerase chain reaction (PCR) and low density microarrays.

Patients, Materials and Methods

Patients

Pediatric patients, aged between 7 days and 15 years, hospitalized for respiratory infections were included in a prospective study conducted from February 1, 2009 until December 31, 2010 in the Pediatric Department of Hospital de Mar, Barcelona. The basic inclusion criterion was an initial diagnosis of (a) respiratory tract infection (rhinopharyngitis, laryngitis, bronchitis or pneumonia), with clinical signs suggesting viral infection; (b) influenza-like syndrome; (c) suspected pertussis; or (d) pneumonia with clinical signs suggesting bacterial infection or empyema, poor progress during admission and suspected viral coinfection.

Personal and epidemiological data were collected together with the reason for admission and clinical progress, duration of hospitalization, oxygen requirements in cases with O_2 saturations $\leq$92%, and need for admission to the Pediatric Intensive Care Unit (PICU) for mechanical ventilation were recorded.

Cases were stratified by age groups (younger than 12 months, between 1 and 3 years, between 3 and 5 years and older than 5 years), and probable diagnosis at time of admission, days of hospitalization (pooled as less than 4 days, between 5 and 10 days and longer than 10 days), need for oxygen therapy, need for transfer to PICU for mechanical ventilation, number of viruses detected and presence of bacterial coinfection were recorded.

Samples for Virological Study

Nasopharygeal aspirate was collected from all patients during the first 12 h of admission. In patients with clinical suspicion of bacterial pneumonia, a nasopharyngeal aspirate specimen was also obtained at any time when poor progress was suspected to be due to viral or bacterial coinfection and not to a poor response to treatment or virulence of the primary etiological agent. Laboratory tests were performed on the same day that the specimen was obtained, except at weekends. Samples were stored at 2–8 °C until analysis.

Extraction of Nucleic Acids

Nucleic acid (RNA/DNA) extraction was performed using MagnaPure LC, Roche Diagnostics.

Amplification and Detection

The CLART® *PneumoVi* kit from Laboratorios Genómica, Madrid, Spain was used. This method can detect and characterize the 19 most common types and subtypes of human viruses causing respiratory infections, including: adenovirus; bocavirus; coronavirus; enterovirus (echovirus); influenza virus A (human H3N2, human H1N1, B, C and H1N1/2009 subtypes); metapneumovirus (A and B subtypes); parainfluenza virus 1, 2, 3 and 4 (A and B subtypes); rhinovirus; respiratory syncytial virus type A (RSV-A); respiratory syncytial virus type B (RSV-B).

Viruses were detected using a multiplex polymerase chain reaction (PCR) technique after reverse transcription of viral RNA (RT-PCR) for amplification of a specific 120–330 base-pair fragment of the viral genome. The amplified product was visualized using the low-density microarray-based technological platform, CLART® (Clinical Array Technology).

Each amplification tube had an internal control to monitor amplification.

In 2009, detection of the influenza A virus H1N1/2009 was not included in the system, but was added in 2010. Until this time, identification was performed using real-time PCR (RT-PCR) that detected the M2 and HA1 genes, using the kit marketed by Roche.

Statistical Analysis

Data were stored in an Excel-supported database and the statistical analysis was performed using SPSS software. First, a univariate descriptive analysis was performed. Qualitative data were presented by the frequency distribution of percentages in each category. In the analytical statistics phase, the number of viruses detected was analyzed to determine any relationship with days of hospitalization, age and need for oxygen therapy. The association between these factors was investigated using hypothesis testing methods, and proportions were compared using the Pearson's Chi-squared test. Results with P-value <.05 were considered significant.

Results

Samples from 463 pediatric patients admitted to the Pediatrics Department of the Hospital del Mar, Barcelona, for community-acquired respiratory infections were analyzed. Results were positive in 385 (83.15%) samples. Age distribution was as follows: younger than 12 months, 173 (44.94%), from 12 months to 3 years, 143 (37.14%), from 3 to 5 years, 53 (13.77%) and older than 5 years, 16 (4.15%); 44.94% of the cases were under the age of 12. None of the patients had risk factors for respiratory diseases. Clinical diagnoses