Virological and clinical characterization of respiratory infections in children attending an emergency department during the first autumn–winter circulation of pandemic A (H1N1) 2009 influenza virus

A. Pierangeli[1], C. Scagnolari[1], C. Selvaggi[1], K. Monteleone[1], S. Verzaro[1], R. Nenna[2], G. Cangiano[2], C. Moretti[2], P. Papoff[2], G. Antonelli[1] and F. Midulla[2]

1) *Virology laboratory, Department of Molecular Medicine and* 2) *Department of Paediatrics, Sapienza University, Rome, Italy*

Abstract

To characterize respiratory virus infections during the first autumn–winter season of pandemic A (H1N1) 2009 influenza virus (A/H1N1/2009) circulation, a prospective study in children attending a paediatric emergency department at the Sapienza University hospital, Rome, was conducted from November 2009 to March 2010. By means of both nasal washings and pharyngeal swabs, enrolled children were checked for 14 respiratory viruses. The majority of acute respiratory infections resulted from viral pathogens (135/231, 58%). Overall, the most common was respiratory syncytial virus (RSV), in 64% of positive samples; A/H1N1/2009 was the only influenza virus found in 16% and rhinovirus (RV) in 15%. Virus-positive children did not differ significantly from virus-negative children in signs and symptoms at presentation; of the virus groups, RSV-infected children were younger and more frequently admitted to intensive-care units than those infected with A/H1N1/2009 and RV. Of the hospitalized children, stratified by age, both infants and children aged >1 year with RSV were most severely affected, whereas A/H1N1/2009 infections were the mildest overall, although with related pulmonary involvement in older children. Children with RV infections, detected in two flares partially overlapping with the A/H1N1/2009 and RSV peaks, presented with bronchiolitis, wheezing and pneumonia. Leukocytosis occurred more frequently in RV-infected and A/H1N1/2009-infected children, and numbers of blood eosinophils were significantly elevated in RV-infected infants. Given the fact that clinical and epidemiological criteria are not sufficient to identify viral respiratory infections, a timely virological diagnosis could allow different infections to be managed separately.

Keywords: Influenza A (H1N1) 2009 virus, influenza-like illness, molecular diagnosis, respiratory syncytial virus, respiratory viruses, rhinovirus

Original Submission: 21 December 2010; **Revised Submission:** 5 April 2011; **Accepted:** 24 May 2011

Editor: R. Charrel

Article published online: 31 May 2011

Clin Microbiol Infect 2012; **18:** 366–373

10.1111/j.1469-0691.2011.03590.x

Corresponding author: G. Antonelli, Department of Molecular Medicine, Sapienza University, Viale di Porta Tiburtina 28, 00185 Rome, Italy

E-mail: guido.antonelli@uniroma1.it

Introduction

Hospital emergency departments (EDs) experience pressure on admissions during the winter months, because of respiratory infections; in winter, various respiratory pathogens that present with similar symptoms circulate widely in children [1]. Diagnosis of influenza virus infections, as with other respiratory pathogens, is usually based on clinical and epidemiological criteria, and is only rarely confirmed virologically [2]. However, lack of an aetiological diagnosis could hamper efforts to reduce the risk of pathogen transmission and the efficient use of the proper antiviral drugs. In order to evaluate the relative impact of a novel virus, laboratory-confirmed data are needed on hospitalization rates for and clinical courses of respiratory viral infections.

In Italy, as in other northern hemisphere countries, pandemic A (H1N1) 2009 influenza virus (A/H1N1/2009) caused a first peak of infection in the spring and early summer months of 2009, and there was a second flare during the autumn, the normal epidemic period for various respiratory viruses. Recent papers have focused on the clinical course of A/H1N1/2009 infection [3–5], comparing patient data from the paediatric population

©2011 The Authors
Clinical Microbiology and Infection ©2011 European Society of Clinical Microbiology and Infectious Diseases

with those available for seasonal influenza infections, but not with other respiratory virus clinical presentations and courses. In only one prospective study [3], conducted in the southern hemisphere, were the clinical features of A/H1N1/2009 compared with those of several other respiratory viruses (although not rhinovirus (RV) and other recently identified viruses).

The fact that A/H1N1/2009 circulates concurrently with other respiratory agents points to a need for better data on the epidemiology, clinical presentation and course of infections caused by the different respiratory viruses.

Accordingly, with the aim of characterizing respiratory viral infections during the first autumn–winter season characterized by A/H1N1/2009 circulation, children attending a paediatric ED in Rome for acute respiratory infections, whether subsequently hospitalized or not, were prospectively enrolled between November 2009 and March 2010. The signs and symptoms, laboratory data and clinical outcomes were then compared in order to characterize A/H1N1/2009 from other respiratory virus infections.

Materials and Methods

Patients

Between November 2009 and March 2010, consecutive children (age 0–16 years) attending the paediatric ED of a teaching hospital (Policlinico Umberto I, 'Sapienza' University of Rome, one of the largest EDs in Rome, with approximately 30 000 consultations per year) were eligible for enrolment in the study, if they presented with fever at admission (temperature ≥38°C) or in the preceding days and/or with at least one acute respiratory symptom (rhinorrhoea, cough, wheezing, or respiratory distress). Exclusion criteria were underlying chronic diseases (including cystic fibrosis, chronic pulmonary disease, congenital heart disease, and immunodeficiency). Informed consent was sought from the children's parents for them to participate in the study, which had been approved by the hospital's Ethics Committee. To ensure accurate virological diagnosis, two respiratory specimens—a pharyngeal swab (PS) (Virocult; Medical Wire and Equipment, Corsham, UK) and a nasal washing (NW) (3 mL of sterile saline solution injected into each nostril and collected with a syringe)—were taken during ED consultation from enrolled children, whether subsequently hospitalized or not. Detailed demographic, clinical and laboratory data were obtained from the patients' medical files. A severity score ranging from 0 to 8 was assigned according to respiratory rate (age-adjusted), arterial oxygen saturation in room air (0, >95%; 1, 95–90%; 2, <90%), the presence of retractions (0, none; 1, present;; 2, present + nasal flare), and ability to feed (0, normal; 1, reduced; 2, intravenous) [6].

PCR-based test for respiratory virus detection

Within 24 h of arrival at the virology laboratory, aliquots of the PS medium (140 μL) that had been collected were used for RNA extraction with a QIAamp Viral RNA Mini Kit (Qiagen, Santa Clara, CA, USA), and subjected to reverse transcription driven by random hexamers, with the High-Capacity cDNA Archive Kit (Applied Biosystems, Monza, Italy). NW samples were vortexed to shear mucus, and divided into three aliquots, one of which was used for RNA extraction as described above. Detection of the influenza A virus was performed with a PCR assay targeting the influenza M gene, according to WHO protocols (http://www.who.int/csr/resources/publications/swineflu/diagnostic_recommendations/en/index.html). Positive samples were subjected to a further PCR reaction, in nested format, targeting the M genomic sequence specific for A/H1N1/2009, with primer pairs designed for this study (first round—MEXF5, 5′-TGCATGGGCCTCATATATACAA-3′, and MEXR5, 5′-ATGACCATCGTCAACATCCA-3′; second round—SM670, 5′-AGCTCCAGTGCTGGTCTGAAAG-3′, and SMR900, 5′-GACTCAGGCACTCCTTCCGTAGAA-3′), and confirmed by sequencing. Thirteen respiratory viruses other than influenza A virus (influenza B virus, respiratory syncytial virus (RSV), adenovirus, RV, parainfluenza viruses (PIVs)1–3, human metapneumovirus (hMPV), coronaviruses OC43, 229E, NL63 and HKU1, and human bocavirus (hBoV)) were tested for with PCR assays, as previously described [7,8]. A case was defined as positive when at least one of each type of sample (NW and PS) gave a positive result.

Quantitative real-time PCR technique for A/H1N1/2009

Viral genome quantification was performed on cDNA from the most A/H1N1/2009-positive PS sample. Primers and probes targeting the influenza M gene were derived from CDC protocols (http://www.who.int/csr/resources/publications/swineflu/CDCrealtimeRTPCRprotocol_20090428) and adapted for use in a 5′-exonuclease TaqMan-based real-time PCR technique. The standards were obtained by cloning 105 bp of the viral M gene into the pCR2.1 plasmid with a TOPO TA cloning kit (Invitrogen, San Diego, CA, USA), according to the manufacturer's instructions. A linear distribution (r = 0.99) was obtained between 10^1 and 10^9 copies of standard plasmid. Data were expressed as number of copies/mL of PS; limits of detection were 10 viral copies/mL.

Statistical analysis

Patient age, number of days of hospitalization and severity score were reported as median (range), and data were compared by means of the Kruskal–Wallis test. The Fisher exact test was used to analyse categorical independent variables.

©2011 The Authors

Download English Version:

https://daneshyari.com/en/article/6131132

Download Persian Version:

https://daneshyari.com/article/6131132

Daneshyari.com