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Short communication

Prospective study of human metapneumovirus infection: Diagnosis, typing and virus quantification in nasopharyngeal secretions from pediatric patients

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Abstract

Study design: During the winter–spring seasons 2004–2005 and 2005–2006, 965 nasopharyngeal aspirates from 871 patients were examined for human metapneumovirus (hMPV) by both monoclonal antibodies (MAbs) and reverse transcription (RT)-PCR.
Results: Overall, 46 samples (4.8%) from 37 patients were positive for hMPV. Of these, 39 were positive by RT-PCR, and 35 by MAbs. Thus, using RT-PCR as a reference assay, the sensitivity, specificity, positive and negative predictive values of MAbs were 71.8%, 99.2%, 80.0% and 98.8%, respectively. Typing showed that concordant results were obtained in 32/46 (69.6%) strains (five untyped), whereas three strains were typed by MAbs only, and 11 by RT-PCR only. Finally, quantification of hMPV RNA allowed to correlate high viral load in nasopharyngeal secretions with acute respiratory symptoms in a group of 11 infants with acute lower respiratory tract infection examined upon admission and discharge from hospital, and a group of nine infants examined upon admission only. Conversely, hMPV etiology was questioned in a group of 14 infants with low viral load.
Conclusions: MAbs may represent an alternative to or a complement to RT-PCR for detection and typing of hMPV strains, while hMPV RNA quantification may help in associating viral load with clinical symptoms.

Keywords: Human metapneumovirus; Acute viral respiratory infections; RT-PCR; hMPV RNA quantification

1. Introduction

Human metapneumovirus (hMPV) is responsible for a significant proportion of respiratory infections in early infancy and childhood (Boivin et al., 2003; Foulongne et al., 2006; Peiris et al., 2003; Williams et al., 2004). Diagnosis of hMPV infections is currently done by reverse transcription (RT)-PCR (van den Hoogen et al., 2004). Virus isolation in cell culture is much less extensively used (Boivin et al., 2002; Chan et al., 2003; Deffrasnes et al., 2005; Williams et al., 2004). Recently, monoclonal antibodies (MAbs) were developed in our laboratory for diagnosis of hMPV infection (Gerna et al., 2006; Percivalle et al., 2005).

In this study, we investigated prospectively: (i) the diagnostic value of MAbs versus RT-PCR in detecting hMPV in nasopharyngeal aspirates (NPA) from young patients with acute respiratory tract infections (ARTI); (ii) the diagnostic value of hMPV typing by MAbs in comparison with RT-PCR; (iii) the discriminatory potential of hMPV quantification in identifying the hMPV etiologic role in ARTI.

Abbreviations: hMPV, human metapneumovirus; MAbs, monoclonal antibodies; ARTI, acute respiratory tract infections; RT-PCR, reverse transcription-PCR; NPA, nasopharyngeal aspirate

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2. Patients and methods

2.1. Viruses, cells and MAbs

All reference and field hMPV strains were recovered and propagated in LLC-MK2 cell cultures (Gerna et al., 2005). hMPV-specific MAbs for virus detection and typing were reported (Percivalle et al., 2005; Gerna et al., 2006). Both hMPV prospective detection and typing were performed in parallel on both NPA cell smears and LLC-MK2 cells from shell-vial cultures (SVC) fixed and stained 48 h postinoculation.

2.2. RT-PCR for hMPV detection and sequencing

Primers for qualitative detection of hMPV types A and B (genes N and F) were originally designed based on GenBank published sequences, as reported (Gerna et al., 2005).

2.3. Phylogenetic analysis

Types and subtypes of hMPV strains were classified by the phylogenetic analysis of two fragments of genes F (nt 661–1094) and N (nt 121–562) (Gerna et al., 2005).

2.4. Quantification of hMPV RNA by RT-PCR

hMPV RNA was quantified by a single-step RT-PCR, as reported (Gerna et al., 2006). A plasmid containing the PCR product of a reference hMPV strain cloned in TA-Cloning (Invitrogen) was serially diluted in the range of 10^5 to 10^0 input copies to construct external reference standards. In addition, an internal RNA control was inserted to achieve more precise quantification. This control consisted of a predetermined amount of a synthetic heterologous RNA sequences unlikely to be present in clinical samples (Armored RNA®, Ebola Virus, subtype Zaire, Ambion RNA Diagnostics, Ambion Inc., Austin, TX). Armored RNA mimicking virions were extracted in parallel with clinical samples and plasmid serial dilutions, and a fixed amount of extracted RNA (100 copies) was added to both reaction mixtures (samples and plasmids) containing, besides the primer pair specific for hMPV, Ebola-specific primers ArmEBOFor (10 μM) and ArmEBORev (10 μM) (Sanchez et al., 1999). The thermal profile was adjusted to achieve maximal sensitivity for both targets (hMPV and Ebola virus). The sensitivity of the assay was 10 DNA copies for both hMPV types A and B.

2.5. Patients

From November 2004 through May 2005 and from November 2005 through May 2006, 965 NPA samples from 871 patients (573 infants and 298 adults) were examined prospectively for hMPV and the other known respiratory viruses (Sarasini et al., 2006).

NPAs were collected from all individuals hospitalised with respiratory symptoms upon admission to hospital and (when indicated) during follow-up, and divided into four aliquots for RT-PCR, direct fluorescent antibody (DFA) staining, SVC inoculation, and storage as a back-up sample, respectively (Rovida et al., 2005).

2.6. Statistics

Comparisons of medians were done by using the Mann–Whitney *U*-test. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of DFA in relation to RT-PCR were calculated based on a composite of DFA results by NPA cell smear and SVC staining.

3. Results

3.1. Incidence of hMPV infections in the hospitalized patient population

Thirty-seven patients (33 infants and 4 adults) out of 871 (4.2%) were found to be positive for hMPV infection, either as a single infection ($n = 24$) or coinfection ($n = 13$).

3.2. HMPV detection by RT-PCR and MAbs

Overall, 46/965 (4.8%) NPAs examined for respiratory viruses were found to be positive by either RT-PCR or MAbs on NPA cell smears or inoculated LLC-MK2 cell spots. Of the 46 hMPV-positive NPAs, 39 were found to be positive by RT-PCR, 26 by DFA, 26 by SVC, and 35 by DFA + SVC (Table 1). Thus, using RT-PCR as a reference assay, the sensitivity, specificity, PPV and NPV of MAbs (DFA + SVC)

Table 1
Comparison of DFA staining of NPA cells and shell-vial cultures vs. RT-PCR for hMPV detection in 965 NPAs from hospitalized patients with acute respiratory tract infections

RT-PCR	No. of samples (%)				Total
	DFA pos SVC pos	DFA pos SVC neg	DFA neg SVC pos	DFA neg SVC neg	
Positive	15 (88.2)	8 (88.9)	5 (55.6)	11 (1.2)	39 (4.0)
Negative	2 (11.8)	1 (11.1)	4 (44.4)	919 (98.8)	926 (96.0)
Total	17 (1.8)	9 (0.9)	9 (0.9)	930 (96.4)	965

RT-PCR, reverse transcription-PCR; DFA, direct fluorescent antibody staining; SVC, shell-vial culture; neg, negative; pos, positive.