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Comparison of four nasal sampling methods for the detection of viral pathogens by RT-PCR—A GA²LEN project☆

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A B S T R A C T

The aim of this study was to compare the efficacy and patient discomfort between four techniques for obtaining nasal secretions. Nasal secretions from 58 patients with symptoms of a common cold, from three clinical centers (Amsterdam, Lodz, Oslo), were obtained by four different methods: swab, aspirate, brush, and wash. In each patient all four sampling procedures were performed and patient discomfort was evaluated by a visual discomfort scale (scale 1–5) after each procedure. Single pathogen RT-PCRs for Rhinovirus (RV), Influenza virus and Adenovirus, and multiplex real-time PCR for RV, Enterovirus, Influenza virus, Adenovirus, Respiratory Syncytial Virus (RSV), Parainfluenza virus, Coronavirus, Metapneumovirus, Bocavirus and Parechovirus were performed in all samples. A specific viral cause of respiratory tract infection was determined in 48 patients (83%). In these, the detection rate for any virus was 88% (wash), 79% (aspirate), 77% (swab) and 74% (brush). The degree of discomfort reported was 2.54 for swabs, 2.63 for washes, 2.68 for aspirates and 3.61 for brushings. Nasal washes yielded the highest rate of viral detection without excessive patient discomfort. In contrast, nasal brushes produced the lowest detection rates and demonstrated the highest level of discomfort.

1. Introduction

Upper respiratory tract infections are the most common cause of acute physical illness in the developed world and the observation that they are followed by acute asthma exacerbations has been known for a long period of time (Lambert and Stern, 1972; Sluder, 1919). However, it was only with the advent of reverse transcription polymerase chain reaction (RT-PCR) detection methods that it was confirmed that the presence of Rhinovirus (RV) and other respiratory viruses may be associated with 80–85% of asthma exacerbations in children, and more than 50% in adults (Johnston, 1995; Nicholson et al., 1993). It is still unknown whether the remaining 15–50% cases are exclusively due to non-viral factors, or that there are still methodological issues in viral detection (Papadopoulos et al., 2003). Furthermore, upper respiratory tract infections in childhood are associated with complications such as otitis media (Chantzi et al., 2006), sinusitis (Pitkaranta et al., 1997), pneumonia (Tsolia et al., 2004) and acute bronchiolitis (Papadopoulos et al., 2002; Xepapadaki et al., 2004). Confirmation of a viral aetiology for respiratory infections is important both for clinical diagnosis as antiviral treatments are becoming available, and for studying respiratory viruses and their interaction with the respiratory tract (Hayden, 2004). Successful detection of a respiratory virus depends on many variables, including sampling for nasal secretions, which may considerably influence the detection rates (Ahluwalia et al., 1987; Barnes et al., 1989; Covalciuc et al., 1999; Frayha et al., 1989; Heikkinen et al., 2001, 2002; Xiang et al., 2002). Several recent studies have attempted to compare different nasal sampling methods (usually no more than two), using mainly detection methods other than PCR, without reaching a clear conclusion (Ahluwalia et al., 1987; Barnes et al., 1989; Covalciuc et al., 1999; Frayha et al., 1989; Heikkinen et al., 2001, 2002; Xiang et al., 2002). Furthermore, nasal sampling can be unpleasant, reducing cooperation especially

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in epidemiological studies that require repeated sampling; this aspect has not been studied before. We hypothesized that there might be significant differences between sampling methods in both virus detection rates and patient acceptance.

The present study aimed to compare the efficacy and degrees of patient discomfort of four different techniques for obtaining nasal secretions, for the determination of respiratory viruses by RT-PCR.

2. Materials and methods

2.1. Patients

This was a prospective multicenter study that took place in Amsterdam, the Netherlands ("center A"), Oslo, Norway ("center B") and Lodz, Poland ("center C"). Participation in the study was offered to patients with recent (≤3 days) symptoms of a common cold, confirmed by physician diagnosis. The study size had been calculated, based upon a power of 0.8, a significance level of 0.05, and an expected differential detection between 70% and 90% in paired samples, to be 53 patients. In total, 58 patients (60% female, age range 7–89 years, median 35 years, mean 39.2 years) were enrolled in the study, after obtaining informed consent. Centers A, B and C recruited 20, 18 and 20 patients, respectively. The study design was approved by the local Ethics Committees of the relevant Institutions. Demographic characteristics were assessed with the use of a standardized questionnaire.

2.2. Sampling methods

Four samples were obtained from the upper respiratory tract of each patient, in the following order: (i) nasal swab, (ii) nasal aspirate, (iii) nasal wash, and (iv) nasal brush, using one nostril for each procedure and alternating nostrils, with an interval of 5–10 min. A nasal swab sample (i) was obtained with a cotton tip (MW104, Medical Wire & Equipment, UK), rubbing the middle meatus. The cotton tips were washed twice in 1 ml of normal saline, spun for 10 min at 400 g and stored at −80 °C. (ii) A nasal aspirate was taken using a sterile mucus trap connected to gentle wall suction. If there was obvious mucus present, the trap was inserted slowly into the nostril and moved slowly in and out while sucking the mucus. A total of 0.5–1 ml of mucus and 5 ml of sterile normal saline, used to wash all the material from the tubing, was obtained. (iii) Nasal washes were performed after 2.5 ml of normal saline were instilled in one nostril (older patients were asked to avoid swallowing). The mucus was harvested 30 s later, using a sterile mucus trap connected to gentle wall suction. The aspirates and washes were placed on wet ice and stored at −80 °C as soon as possible. (iv) Nasal brushings were harvested from the nasal cavity with a brush (Cytobrush Plus, Medscand Medical, Sweden) by sampling the middle meatus. The brushes were washed twice in 1 ml of normal saline, spun for 10 min at 400 g and stored at −80 °C.

2.3. RNA isolation, quantitation and RT-PCR

Viral detection was performed independently in two laboratories. In the Allergy Research Center, 2nd Department of Pediatrics, University of Athens, single pathogen RT-PCRs were performed for the detection of RV, Influenza virus and Adenovirus. RNA was extracted using Trizol (Invitrogen, CA, USA), according to the manufacturer's recommendations. Two-microliter aliquots of the isolated RNA were diluted in Tris–Cl pH 7.5 and RNA yield, concentration and purity were determined spectrophotometrically using an Eppendorf BioPhotometer (Hamburg, Germany). Reverse transcription (cDNA synthesis) was performed in 20 μl reactions using 8 μl RNA, Superscript III reverse transcriptase (Invitrogen) and random hexamers according to the manufacturer's instructions.

RT-PCRs were done in 50 μl reactions consisting of 1x Buffer, 3 mM Mg^{2+}, 0.2 mM dNTPs, 2U of Platinum Taq DNA polymerase (Invitrogen), and 0.2 μM of each primer. PCR for RV was done with 6 μl cDNA and OL26 and OL27 primers (Papadopoulos et al., 2000). PCR for Influenza virus (serotypes AH1, AH3, B) was done in two rounds (nested-PCR); in the first round mixture 4 μl of cDNA were added and 2 μl of primary product were then transferred to 48 μl of the secondary amplification mixture using a second primer set internal to that of the first round (Stockton et al., 1998). PCR for Adenovirus was done with 4 μl of cDNA in a single round, according to Freymuth et al. (1997). Samples were amplified in a PTC-200 DNA Engine thermocycler (MJ Research, MA, USA), with an initial denaturation step at 94 °C for 2 min and then under conditions described in Table 1.

A real-time Taqman multiplex PCR assay was performed in AMC, Dpt Medical Microbiology, Amsterdam, for RV, Enterovirus, Influenza virus, Adenovirus, RSV, Parainfluenza virus, Coronavirus, Metapneumovirus, Bocavirus and Parechovirus as described before (Molenkamp et al., 2007).

Patients positive for viral agents ("infected") were defined as positive for any virus by any of the used methods, and negative ("uninfected") as those negative for all the viruses and by all methods simultaneously.

The patients' discomfort was assessed using a visual rating scale (range: 1–5). The patient was asked to choose the face that best describes how he/she was feeling with each procedure.

2.4. Statistics

Statistic analysis was performed by chi-square, Wilcoxon Signed Ranks, Kruskal–Wallis and Mann–Whitney tests using SPSS v.13 software. A p value <0.05 was regarded as significant.

3. Results

3.1. RNA quantitation

The extracted RNA concentration was 312.54 ± 44.51 μg/ml for the aspirates, 279.66 ± 43.61 μg/ml for the brushes 306.05 ± 50.32 μg/ml for swabs and 330.18 ± 42.99 μg/ml for the washes (non-significant differences). Purity ($A_{260/280nm}$) was consistent and ranged from 1.79 to 1.92.

3.2. Viral detection

In 48 out of 58 patients (83%), at least one type of virus was detected, by any of the four methods. Rhinovirus was found in 39 patients (67%), Adenovirus in 15 (26%), Influenza virus in 11 (19%), Coronavirus in 6 (10%), Parainfluenza virus in 3 (5%) and Bocavirus in 1 patient (2%). Detailed detection rates are presented in Table 2. The agreement between single pathogen RT-PCR and Taqman multiplex real-time PCR was 80% for RV, 94% for Influenza virus and 91% for Adenovirus. Nasal wash samples identified 88% of the infected patients, which was the highest detection rate. Aspirates detected 79% of the infected patients, swabs 77% and brushes 74%. The rate of detection of any virus in nasal washes was significantly higher than that in nasal brushes ($p < 0.05$) (Table 2).

3.3. Individual virus analysis

Comparing the detection rates of the four methods regarding the virus type, nasal washes yield the highest detection rates for