Journal of Clinical Virology 79 (2016) 12–17

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Diagnostic performance of influenza viruses and RSV rapid antigen detection tests in children in tertiary care

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ARTICLE INFO

Article history:
Received 3 February 2016
Received in revised form 22 March 2016
Accepted 24 March 2016

Keywords:
Rapid antigen detection tests
Paediatrics
Influenza
Respiratory syncytial virus
Tertiary care

ABSTRACT

Background: Rapid antigen detection tests (RADTs) are increasingly used to detect influenza viruses and respiratory syncytial virus (RSV). However, their sensitivity and specificity are a matter of debate, challenging their clinical usefulness.
Objectives: Comparing diagnostic performances of BinaxNow Influenza AB® (BNI) and BinaxNow RSV® (BNR), to those of real-time reverse transcriptase PCR (RT-PCR), virus isolation and direct immunofluorescence (D-IF) in paediatric patients.
Study design: Between November 2005 and September 2013, 521 nasal washings from symptomatic children (age <5 years) attending our tertiary care centre were tested, with a combination of the respective assays using RT-PCR as gold standard.
Results: Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of BNI were 69% (confidence interval [CI] [51–83]), 96% [94–97], 55% [39–70] and 98% [96–99] respectively. Of eleven false-negative samples, RT-PCR Ct-values were higher than all RT-PCR positive test results (27 vs 22, $p = 0.012$). Of twenty false-positive samples, none were culture positive and two tested positive in D-IF.

Sensitivity, specificity, PPV and NPV for BNR were 79% [73–85], 98% [96–99], 97% [93–99] and 88% [84–91]. Of the 42 false-negative samples the median Ct-value was higher than that of all RT-PCR positive samples (31 vs 23, $p < 0.0001$). Five false-positive samples were detected. Three of these tested positive for RSV in virus isolation and D-IF.
Conclusions: RADTs have a high specificity with BNR being superior to BNI. However, their relative low sensitivity limits their usefulness for clinical decision making in a tertiary care paediatric hospital.

1. Background

Influenza viruses and respiratory syncytial viruses (RSV) cause acute respiratory tract infections (ARTIs) in children, being a leading cause of hospitalization [1–3]. Identification of both viruses is important for disease management, as the presence of these infections may require specific treatment (i.e. oseltamivir) and hospital containment measures. The current gold standard for detection of these viruses is real-time reverse transcriptase PCR (RT-PCR) [4]. This is however not performed in all hospitals, as it requires a molecular diagnostic laboratory with specialized personnel and equipment. Instead, rapid antigen detection tests (RADTs) are used as these assays are easier and cheaper to perform and less time-consuming [5–7]. The performance of these tests depends on factors like time between disease onset and sampling, quality and type of specimen and epidemiological parameters [8]. Diagnostic value and clinical usefulness of RADTs for influenza diagnosis vary greatly [5–7,9–12]. This prompted us to evaluate the diagnostic performance of the routinely used RADTs (manufactured by Alere BinaxNOW®) for these two viruses as used in our tertiary care paediatric hospital.

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http://dx.doi.org/10.1016/j.jcv.2016.03.022

2. Objectives

Comparing diagnostic performances of two RADTs, BinaxNow Influenza AB® (BNI) and BinaxNow RSV® (BNR), with those of RT-PCR in samples of paediatric patients attending our tertiary care centre with ARTIs for a period of almost eight consecutive years. Discrepant data were subsequently compared with those of virus isolation and direct immunofluorescence (D-IF) assays.

3. Study design

This study was conducted from November 2005 through September 2013, we identified paediatric patients between 0 and 5 years who attended Erasmus MC-Sophia's emergency department, out-patient-clinic and those who were hospitalized in this period. To analyse the performance of the BNI and BNR compared to RT-PCR we selected 521 nasal washings of 489 patients with a median age of 4 months (minimum 0.03–maximum 58 months, lower interquartile range 1.6–upper interquartile range 9.8) and 55% (268/489) were male. Nasal washings were obtained during routine clinical practice in symptomatic children and were tested immediately after sampling by trained laboratory personnel using all four diagnostic methods. Multiple samples from the same patient were included in our analysis. Therefore patients are referred to as cases. Data regarding gender, age and hospital admission were obtained from the electronic patient files.

3.1. Ethics

Data collection and analyses were conducted on anonymized samples, which does not require further medical ethics review as consented by our Medical ethical board (MEC-2015-306).

4. Tests

4.1. RT-PCR gold standard

All nasal washings were tested for the presence of selected viruses by means of RT-PCR with primers and probes sets used in the routine setting of our department [13]. In short, RNA and DNA were extracted using MagnaPureLC (Roche Diagnostics, Almere, the Netherlands) and the total nucleic acid isolation kit. The extractions were internally controlled by addition of a known concentration of phocine distemper virus (PDV) and phocine herpes virus (PHV). Uni-plex RT-PCR was used to detect RSV-A, RSV-B, human rhinovirus (HRV), parainfluenza virus (PIV) type 3 (PIV-3), adenovirus (ADV), and human bocavirus (HBoV). Duplex reactions were performed combining influenza A virus and PDV, influenza B virus and human coronavirus (HCoV) OC43 (HCoVOC43), human metapneumovirus (HMPV) and PIV-2, HCoV229E and PIV-4, and HCoVNL63 and PIV-1. A cycle threshold value (Ct-value) of <40 was defined positive for any virus. RT-PCRs were developed in-house for influenza viruses and RSV-A and validated [13]. RSV-B primers and probes were used as reported by Dewhurst-Maridor et al. [14].

4.2. Rapid antigen detection tests (RADTs)

Alere BinaxNOW® Influenza A and B (BNI) and Alere BinaxNOW® RSV (BNR) (Scarborough, Maine, USA) are commercially available in vitro immunochromatographic assays for the qualitative detection with monoclonal antibodies directed against influenza A and B virus nucleoproteins and RSV fusion protein antigen, respectively. Nasal washings were obtained using standard protocols and rapid antigen testing was performed as described by the manufacturer. For our analyses the test results of BNI influenza A and influenza B were combined into a single influenza BNI dataset as influenza B was not encountered frequently with only four influenza B BNI positive samples, two of which were influenza B RT-PCR positive.

4.3. Virus isolation assay

Virus isolation assays were always performed in combination with D-IF. Madin-Darby Canine Kidney (MDCK) cell line (NBL-2) (ATCC® CCL-34™) and the human cell line HEp-2 (ATCC® CCL-23™) were used to isolate influenza viruses and RSV respectively. Virus cultures were regularly checked for cytopathic effect by light microscopy. Immunofluorescence with fluorescein isothiocyanate (FITC) labeled monoclonal antibodies was used to confirm the presence of influenza virus or RSV [15].

4.4. Direct immunofluorescence (D-IF) assays in clinical specimens

Cells were isolated from nasal washings, dried on microscope slides, and fixed with acetone. Subsequently, cells were stained with FITC conjugated monoclonal antibodies against influenza A virus, influenza B virus or RSV (IMAGEN™ Influenza A and B and IMAGEN™ RSV, Hampshire, United Kingdom). Specimens were incubated with FITC-conjugated antibodies for 15 min at 37 °C, subsequently excess reagent was washed off with phosphate buffered saline. The stained area was then mounted and viewed by fluorescent microscopy.

4.5. Comparison between tests

The focus of our study was to compare data obtained with two RADTs, BNI and BNR with those obtained by RT-PCR as gold standard. We defined false-negative tests as those for which the rapid test was negative and the gold standard RT-PCR positive; a false-positive test result was defined if the rapid test tested positive and the gold standard RT-PCR tested negative. We compared the available Ct-values in all respective categories of samples and analysed whether there was an association between Ct-values and RADTs results and hospitalization. For influenza all Ct-values were available, for RSV Ct-values were available for 183/204 (90%) of the performed tests. Missing Ct-values were from samples tested in 2005 and 2006 when routine input of Ct-values in our laboratory system was not yet performed and digital documentation was not available. Finally, false-negative and false-positive test results were compared to test results obtained with the other virus detection methods: virus isolation and D-IF assays.

4.6. Statistical analyses

The main outcomes of this study were the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the BNI and BNR rapid test results compared to RT-PCR during the total study period and during viral season (October 1st through March 31st). Ct-values were compared with Mann-Whitney *U* tests.

5. Results

5.1. Sensitivity and specificity of BNI

Of 521 nasal washings both influenza RT-PCR and BNI data were available. Most were obtained between September and March (see Supplemental data Figs. S1 and S2 in the online version at doi: 10.1016/j.jcv.2016.03.022). Of these, 35 cases tested positive with RT-PCR (35/521, 7%, median Ct-value 22 [range] [17–39]) whereas 44 tested positive in the BNI (44/521, 8%). Of the 35 RT-PCR positive