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Impact of PCR for respiratory viruses on antibiotic use: theory and practice

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Impact of PCR for respiratory viruses on antibiotic use: theory and practice

Alma C. van de Pol, MD, PhD^{ab}, Tom F.W. Wolfs, MD, PhD^a, Carline E.A. Tacke^a, Cuno S.P. Uiterwaal, MD, PhD^c, Johannes Forster, MD, MME^d, Anton M. van Loon, PhD^b, Jan L.L. Kimpen, MD, PhD^a, John W.A. Rossen, PhD^{be}, Nicolaas J.G. Jansen, MD, PhD^f

^aDepartment of Pediatrics, Division of Infectious Diseases

^bDepartment of Virology

^cJulius Center for Health Sciences and Primary Care

^fDepartment of Pediatrics, Division of Intensive Care

University Medical Center Utrecht

Heidelberglaan 100, 3584 CX Utrecht, The Netherlands

^dDepartment of Pediatrics

St. Josefskrankenhaus and University Hospital Freiburg

Sautierstrasse 1

D-79104 Freiburg, Germany

^eLaboratory of Medical Microbiology and Immunology

St. Elisabeth Hospital Tilburg

Hilvarenbeekseweg 60, 5022 GC Tilburg, The Netherlands

Corresponding author:

N.J.G. Jansen

Department of Pediatrics, Room KG.01.319.0

1
2
3 Wilhelmina Children's Hospital, University Medical Center Utrecht
4

5 Lundlaan 6, 3584 EA Utrecht,
6

7
8 The Netherlands
9

10 Phone: #31 88 7554002
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12 Fax: #31 88 7555347
13

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15 Email: n.j.g.jansen@umcutrecht.nl
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20 **Running head**
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23 Impact of respiratory virus real-time PCR
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SUMMARY

Rationale for the study

Real-time PCR for respiratory viruses is more sensitive, yet more expensive, than conventionally used direct immunofluorescence (DIF). We determined the impact of real-time PCR, additional to DIF, on antibiotic prescription in ventilated children with lower respiratory tract infection (LRTI) at admission to the pediatric intensive care unit (PICU).

Methods

First, a multicenter survey study was performed. Subsequently, in a prospective study, children (≤ 5 years) with LRTI were tested at admission by DIF and PCR. Positive DIF results were reported at the end of the first working-day. PICU physicians reported antibiotic treatment on the second working-day. After informing them of the PCR result antibiotic treatment was reevaluated.

Results

The multicenter survey study (94 respondents) showed that PCR decreased antibiotic use ($p < 0.001$). In the prospective study 38 children were included, of which 19 (50%) were DIF positive. Of the 19 DIF-negative patients 12 (63%) were treated with antibiotics before revealing the PCR result; the PCR test was positive in 9 out of 12. Revealing PCR results did not alter antibiotic treatment. In 7 DIF-negative patients not given antibiotics, the PCR test was positive.

Conclusion

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3 In contrast to their responses to the survey study, in real-life PICU physicians did not let their
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5 antibiotic prescription be influenced by respiratory real-time PCR in children ventilated for
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8 LRTI.
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For Peer Review

Keywords

Anti-bacterial agents, viral lower respiratory tract infections, patient care management, pediatric intensive care units, real-time polymerase chain reaction, questionnaires.

Abbreviations:

LRTI: lower respiratory tract infection

PICU: pediatric intensive care unit

DIF: direct immunofluorescence

PCR: polymerase chain reaction

OI: oxygenation index

RSV: respiratory syncytial virus

INTRODUCTION

Lower respiratory tract infection (LRTI) in young children is a common reason for admission to the pediatric intensive care unit (PICU).(1;2) The large majority of LRTI in these children is believed to be of viral origin, in which case antibiotic treatment would be unnecessary. (3;4) However, the differentiation between viral, bacterial or mixed infections is clinically impossible and bacteria can be cultured in a considerable proportion of patients.(5) Therefore most children ventilated with LRTI are treated empirically with antibiotics.(6;7) It is unclear whether the introduction of new rapid respiratory viral tests into the work-up of LRTI impacts antibiotic prescription.(8) The limited number of studies evaluating the effect of rapid diagnosis on antibiotic use yielded contradictory results.(9-14) The impact of viral testing in the PICU setting has not been investigated previously.

Rapid viral testing in the hospital setting has traditionally been performed with direct immunofluorescence tests (DIF). Currently, molecular amplification tests such as real-time polymerase chain reaction (PCR) have become the new golden standard for the detection of respiratory viruses because of their increased sensitivity.(15;16) Real-time PCR assays have been shown to be quick and reliable in the PICU population, leading to a large increase in diagnostic yield of respiratory viruses.(17-20) At present, the high costs of PCR assays are considered a disadvantage.

The aim of our study was to evaluate whether a viral diagnosis obtained by the more sensitive real-time PCR as compared to DIF, would impact antibiotic prescription in children admitted with LRTI at the PICU. To answer this question we first performed a multicenter survey study among PICU physicians. Subsequently, we conducted a 2-year prospective study at our PICU.

MATERIALS AND METHODS

Multicenter survey study

Respondents

A total of six PICU's from three large Dutch medical centers (University Medical Center Utrecht, Amsterdam Medical Center, Erasmus Medical Center Rotterdam) and three large German medical centers (University Medical Center Freiburg, Universitätsklinikum Mainz, Charité Berlin) participated in this multicenter study. A senior pediatric intensivist of each PICU listed the PICU physicians involved in the care of infants with LRTI at their PICU. Physicians were individually approached by the investigator or the pediatric intensivist for a response.

Questionnaire

Questionnaires consisted of five case descriptions, constructed from clinical data from five infants with typical signs of LRTI ventilated at our PICU in 2004. The infants were selected because they had a negative or indeterminate DIF test and a positive real-time PCR result. Characteristics of the five cases are shown in Supplementary table 1. After each case description the following question was asked: "Would you start/continue antibiotic therapy for this child?". Questions were first asked with the real-time PCR result concealed, and the same questions were repeated after disclosing the real-time PCR result. Anonymity of the respondents was assured. The questionnaire was pilot-tested to assure clarity and coherence, and it was subsequently modified in response to the pilot test results. The order of cases was random in the different questionnaires. Due to the low burden of the survey, ethical approval/informed consent was not needed according to the institutional review board of our hospital.

Prospective study

Patients

Ventilated patients under 5 years old admitted with LRTI to the PICU of the Wilhelmina Children's Hospital, who had a DIF test performed as part of routine investigation, were enrolled from October 2006 through October 2008. The Wilhelmina Children's Hospital is a tertiary care medical center with a 16 bed PICU facility. It serves as a referral center for the central part of the Netherlands. Exclusion criteria were asthma exacerbation, immune compromised state, indication for antibiotics other than LRTI, and repeated PICU admission for LRTI during the study period. Baseline clinical data were obtained from medical charts using standardized forms (gender, age, symptom onset, disease severity, antibiotics before admission, preterm birth, underlying illness, duration of ventilation, length of PICU stay, white blood count and C-reactive protein). Underlying illness was defined as chronic pulmonary disease, congenital heart disease, neurological disease or gastrointestinal disease.(21) For the assessment of illness severity the oxygenation index (OI) was calculated 12 hours after admission ($OI = \text{fraction of inspired oxygen} \times \text{mean airway pressure} \times 100 / \text{partial oxygen pressure}$).

Viral testing

Nasopharyngeal aspirates were taken by trained nursing staff the morning of the first working-day after admission (working-days: Monday through Friday). Specimens were examined with DIF and real-time PCR as previously described.(18;22-24) In short, part of the sample was subjected to DIF assays to detect respiratory syncytial virus (RSV), influenza viruses, parainfluenza viruses 1–3 and adenoviruses using Imagen kits (DaKo, Glostrup, Denmark), in accordance with the manufacturer's recommended protocol. Indeterminate DIF

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3 results were considered negative for the analyses. The remaining material was used for real-
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5 time PCR testing for RSV, influenza viruses, parainfluenza viruses 1-4, adenoviruses,
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7 rhinoviruses, coronaviruses, human metapneumovirus, *Mycoplasma pneumoniae*. During the
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9 second year the newly detected human bocavirus was added to the panel.(25;26) Briefly, after
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11 nucleic acid extraction using the MagNA pure LC nucleic acid isolation system (Roche
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13 Diagnostics, Basel, Switzerland) amplification was carried out in a 25- μ l reaction mixture on
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15 a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The positive
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17 control for human bocavirus was provided by T. Allander, Karolinska Institute, Stockholm,
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19 Sweden. Internal control viruses were used to monitor for efficient extraction and
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21 amplification.
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29 Patient management questionnaire

30 Patient care of enrolled patients was left at the discretion of the attending PICU physician.
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32 Results of the DIF assays had a turn-around time of approximately five hours, and positive
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34 results were reported at the end of the first working-day to the attending clinician. Results of
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36 the real-time PCR assays had a turn-around time between 16 and 24 hours and the results
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38 were reported at the beginning of the second working-day to the investigator only. The
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40 investigator approached the attending physician before revealing the PCR result, using a
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42 questionnaire with the following questions: (1) "What is the patients' suspected clinical
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44 diagnosis?" (To be answered with checkboxes for viral bronchiolitis, viral pneumonia, and
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46 bacterial pneumonia), and (2) "Is the patient treated with antibiotics?". Subsequently, the
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48 physician was provided one hour later with the PCR result and the questions were repeated.
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50 Finally, PICU physicians were asked what the reason was for (dis)continuing antibiotics.
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52 Since the prospective study did not involve a change in patient management, ethical approval
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3 and/or informed consent of parents/guardian of infants was not required according to the
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5 institutional review board of our hospital.
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10 Statistical analysis

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12 For statistical analysis of the percentage of antibiotic use before and after disclosure of the
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14 real-time PCR results the McNemar test was used.
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For Peer Review

RESULTS

Multicenter survey study

Respondents

Ninety-four PICU physicians (20 from Utrecht, 12 from Amsterdam, 16 from Rotterdam, 17 from Freiburg, 12 from Mainz, and 17 from Berlin) completed the questionnaire.

Antibiotic use

Before disclosure of the real-time PCR result, the percentage of PICU physicians that would treat the five infants with antibiotics was 37%-98% (range for the five cases; figure 1). After disclosure of the real-time PCR result the percentage of physicians that would treat the infants with antibiotics decreased to 19%-82% (McNemar test: $p < 0.001$ for all cases). The absolute reduction was largest for case 1 and 5 (37% and 42%). Although the percentage of antibiotic use pre-PCR test differed between centers, the reduction of antibiotic use following PCR was similar in the different centers.

Prospective study

Patients

Thirty-eight children with LRTI were enrolled in this study. Twenty (53%) children were clinically suspected of viral bronchiolitis, 9 (24%) of viral pneumonia and 9 (24%) of bacterial pneumonia. Demographics and clinical characteristics of the enrolled patients are provided in table 1.

Viral test results

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3 Nineteen patients were positive with DIF (50%), all for RSV; no (para)influenza or
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5 adenoviruses were detected (figure 2). Real-time PCR confirmed all 19 positive DIF results
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7 and detected 7 additional viruses in 6 of them (table 2). The remaining 19 patients were DIF
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9 negative. In 16 of the 19 DIF negative patients real-time PCR detected a total of 20 viruses. In
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11 3 (8%) patients with LRTI no virus could be identified either by DIF or by PCR. Therefore,
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13 an infection with one or more viruses was detected in 35 of 38 patients (92%).
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20 Antibiotic use before and after the PCR result

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22 In the group of 19 DIF positive patients, 9 (47%) were treated with antibiotics before the real-
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24 time PCR result was made available. Antibiotic prescription did not change after revealing the
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26 concordant positive real-time PCR results (figure 2).
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29 In the group of 19 DIF negative patients, 12 (63%) patients were treated with antibiotics
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31 before the PCR result was made available. The real-time PCR was positive in 9 of these 12
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33 patients. In none of the 9 patients the positive real-time PCR result prompted the PICU
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35 physicians to discontinue antibiotics. In only 1 of these 9 patients the suspected clinical
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37 diagnosis was changed by the attending physician from bacterial to viral pneumonia,
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39 nevertheless antibiotic treatment was continued. When asked for the reason to continue
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41 antibiotics, physicians listed (a) clinical suspicion of bacterial superinfection (ill-appearance,
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43 high ventilator settings, high C-reactive protein levels; n=7) and (b) waiting for the final result
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45 of the bacterial cultures (n=2). Finally, in the 7 DIF negative patients that were not treated
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47 with antibiotics, a negative real-time PCR result could have prompted physicians to start
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49 antibiotics. However, all 7 patients were found to be positive by real-time PCR for respiratory
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51 viruses; i.e. real time PCR confirmed the clinical suspicion of a viral infection and no
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53 antibiotics were started.
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3 In summary, 21 (55%) of the included 38 patients were treated with antibiotics and this
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5 number was not influenced by the real-time PCR results. Of the 29 patients that were believed
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7 by the PICU physician to have a viral lower respiratory tract infection (viral bronchiolitis or
8
9 viral pneumonia), 12 (41%) had antibiotics prescribed.
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14 15 **DISCUSSION**

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17 The present study investigated the impact of respiratory virus real-time PCR, in addition to
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19 DIF, on antibiotic prescription in ventilated children with LRTI at the PICU, first in a survey
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21 study and then in a prospective study. Although physicians indicated that real-time PCR
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23 would have an effect on antibiotic use in the survey study, this effect was not found in
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25 practice in the prospective study.
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32 A variety of viral tests are currently used in the diagnostic work-up of children with LRTI
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34 at the PICU. Conventional rapid viral tests such as antigen detection by DIF are now being
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36 replaced by more sensitive, molecular assays such as real-time PCR. This has led to an
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38 increase in the detection of viruses,(18;27-29) with a substantial increase in costs.(22) To
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40 determine if real time PCR theoretically could influence daily practice at the PICU, first a
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42 multicenter survey study was performed. Since the survey study indicated that the availability
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44 of PCR result would reduce antibiotic use, a 2-years prospective study was undertaken at a
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46 single center to test the impact of real-time PCR in real-life.
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53 Three limitations of our study deserve further discussion. First, the number of evaluated PICU
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55 children in the prospective study was relatively small. However, real-time PCR did not
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57 influence antibiotic prescription in one single patient. Therefore, we believe that the inclusion
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59 of additional patients is not likely to influence our observation that the impact of real-time
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3 PCR is minor. Second, the fact that physicians were aware of the study may have influenced
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5 their decision on antibiotic prescription. However, this would probably have led to an
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7 overestimation of the effect of real-time PCR results on antibiotic use which would only make
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9 the conclusion of our study stronger. Third, the turn around time of real-time PCR in our
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11 study was between 16 and 24 hours and PCR was performed on working-days, so the results
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13 were available only at the second working-day. Possibly, if results had been available earlier
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15 this could have had more effect on antibiotic prescription practices (physicians will probably
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17 be more reluctant to stop antibiotic treatment than not to start it). However, we aimed to
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19 evaluate the impact of real-time PCR in daily practice, and in most hospitals both DIF and
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21 real-time PCR are only performed on working-days (i.e. Monday-Friday) with the same turn
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23 around times for both tests.(30)
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32 In agreement with previous studies we showed that real-time PCR increased the diagnostic
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34 yield considerably when compared to DIF. The proportion of virus-infected children
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36 increased from 50% (DIF) to 92% (real-time PCR), and the number of viruses from a total of
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38 19 (all RSV) to 46 (8 different types). This increased diagnostic yield was not, however,
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40 accompanied by a decrease in antibiotic prescription. This finding is in contrast with our
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42 survey study in which real-time PCR decreased the percentage of antibiotic description by 16-
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44 42% (absolute reduction; $p < 0.001$ for all cases). Apparently, the treatment of paper patients is
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46 not the same as treating patients in daily PICU practice, in which physicians seem to prefer to
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48 stay on the safe side. In daily practice, physicians seem to make clinical decisions on other
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50 grounds than results from diagnostic investigations. Reasons for not stopping antibiotic
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52 treatment in PCR positive patients were the clinical suspicion of a (secondary) bacterial super
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54 infection, and the fact that the results of bacterial cultures were still unknown. Additional to
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56 the lack of effect of real-time PCR on antibiotic use found in this study, one may wonder what
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3 the effect of any viral test such as DIF may be, since a considerable proportion of patients
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5 (47%) received antibiotics regardless of a positive DIF result.
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10 Previous studies on the impact of rapid viral testing on antibiotic use in children have been
11 published. Our results are in agreement with Doan et al and Poehling et al, who did not find a
12 reduction in antibiotic prescription in children randomized to have rapid testing performed at
13 the emergency department.(10;12) Our results are in contrast with studies from Bonner et al,
14 Noyola et al, Sharma et al, and Woo et al, who found that physicians prescribed less
15 antibiotics in patient groups tested with a rapid viral test.(9;11;13;14) These studies were all
16 performed at the emergency department or at pediatric wards, and not at the PICU, and
17 assessed different age groups. It can be expected that physicians in a PICU setting are more
18 reluctant to withhold antibiotic treatment in the severely ill population with LRTI who
19 frequently has underlying diseases. This is reflected in our population by the finding that in
20 the 38 ventilated LTRI patients 21 children started with antibiotics at admission (55%) and
21 antibiotic prescription was not changed in any of the groups, not even in the 9 children with a
22 positive PCR and negative DIF.
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43 In addition, virtually all LRTI patients at our PICU (35 out of 38; 92%) were found to be
44 positive by real-time PCR for one or more viruses. This means that in our PICU population
45 virus-positivity does not help clinicians anymore to differentiate between those patients who
46 qualify for antibiotics from those who could safely be treated without antibiotics. Therefore,
47 we propose that future real-time PCR studies should be directed to settings where not all
48 patients are found to harbour viruses, such as the population of children with less severe
49 respiratory tract infection in the community.(10)
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3 Limiting antibiotic prescriptions in patients with viral LRTI is not the only objective of
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5 performing respiratory real-time PCR assays. Real-time PCR is valuable as a very sensitive
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7 tool to further explore the clinical presentation and impact of respiratory viruses as well as
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9 their epidemiology and it will become an even more important diagnostic tool when antiviral
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11 treatments become available. However, when real-time PCR in LRTI patients at the PICU
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13 does not affect daily practice, this test could be left out of the diagnostic work-up and costs
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15 could be saved.
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22 In conclusion, in daily practice real time PCR, in addition to DIF testing, has no impact on the
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24 prescription of antibiotics in ventilated LRTI patients at the PICU. This is in contrast to the
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26 response of physicians on paper case patients in a theoretical survey. This study shows a
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28 discrepancy between theoretical considerations and daily practice at the pediatric intensive
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30 care. In daily practice physicians seemed reluctant to stop antibiotic prescriptions in the
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32 severely ill population of LRTI patients at the PICU.
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3 **Figure legends**
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8 **Figure 1**
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10 Title: Antibiotic use before and after disclosure of the real-time PCR results in the survey
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12 study
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15 Subscript: * Difference McNemar test $p < 0.001$
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20 **Figure 2**
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22 Title: Flow-chart of viral test results and antibiotic use of patients enrolled in the prospective
23
24 study.
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26
27 Subscript:
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29 ^aTwelve patients that were negative by direct immunofluorescence were treated with
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31 antibiotics.
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34 ^bNine out of twelve patients that were negative by direct immunofluorescence and treated
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36 with antibiotics were subsequently positive by real-time PCR. These are the patients in which
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38 antibiotic prescription is most likely to be changed.
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Impact of PCR for respiratory viruses on antibiotic use: theory and practice

Alma C. van de Pol, MD, PhD^{ab}, Tom F.W. Wolfs, MD, PhD^a, Carline E.A. Tacke^a, Cuno S.P. Uiterwaal, MD, PhD^c, Johannes Forster, MD, MME^d, Anton M. van Loon, PhD^b, Jan L.L. Kimpen, MD, PhD^a, John W.A. Rossen, PhD^{be}, Nicolaas J.G. Jansen, MD, PhD^f

^aDepartment of Pediatrics, Division of Infectious Diseases

^bDepartment of Virology

^cJulius Center for Health Sciences and Primary Care

^fDepartment of Pediatrics, Division of Intensive Care

University Medical Center Utrecht

Heidelberglaan 100, 3584 CX Utrecht, The Netherlands

^dDepartment of Pediatrics

St. Josefskrankenhaus and University Hospital Freiburg

Sautierstrasse 1

D-79104 Freiburg, Germany

^eLaboratory of Medical Microbiology and Immunology

St. Elisabeth Hospital Tilburg

Hilvarenbeekseweg 60, 5022 GC Tilburg, The Netherlands

Corresponding author:

N.J.G. Jansen

Department of Pediatrics, Room KG.01.319.0

1
2
3 Wilhelmina Children's Hospital, University Medical Center Utrecht
4

5
6 Lundlaan 6, 3584 EA Utrecht,
7

8 The Netherlands
9

10 Phone: #31 88 7554002
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12 Fax: #31 88 7555347
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15 Email: n.j.g.jansen@umcutrecht.nl
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20 **Running head**
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23 Impact of respiratory virus real-time PCR
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For Peer Review

SUMMARY

Rationale for the study

Real-time PCR for respiratory viruses is more sensitive, yet more expensive, than conventionally used direct immunofluorescence (DIF). We determined the impact of real-time PCR, additional to DIF, on antibiotic prescription in ventilated children with lower respiratory tract infection (LRTI) at admission to the pediatric intensive care unit (PICU).

Methods

First, a multicenter **survey** study was performed. Subsequently, in a prospective study, children (≤ 5 years) with LRTI were tested at admission by DIF and PCR. Positive DIF results were reported at the end of the first working-day. PICU physicians reported antibiotic treatment on the second working-day. After informing them of the PCR result antibiotic treatment was reevaluated.

Results

The multicenter **survey** study (94 respondents) showed that PCR decreased antibiotic use ($p < 0.001$). In the prospective study 38 children were included, of which 19 (50%) were DIF positive. Of the 19 DIF-negative patients 12 (63%) were treated with antibiotics before revealing the PCR result; the PCR test was positive in 9 out of 12. Revealing PCR results did not alter antibiotic treatment. In 7 DIF-negative patients not given antibiotics, the PCR test was positive.

Conclusion

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3 In contrast to their responses to the **survey** study, in real-life PICU physicians did not let their
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5 antibiotic prescription be influenced by respiratory real-time PCR in children ventilated for
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8 LRTI.
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Keywords

Anti-bacterial agents, viral lower respiratory tract infections, patient care management, pediatric intensive care units, real-time polymerase chain reaction, questionnaires.

Abbreviations:

LRTI: lower respiratory tract infection

PICU: pediatric intensive care unit

DIF: direct immunofluorescence

PCR: polymerase chain reaction

OI: oxygenation index

RSV: respiratory syncytial virus

INTRODUCTION

Lower respiratory tract infection (LRTI) in young children is a common reason for admission to the pediatric intensive care unit (PICU).(1;2) The large majority of LRTI in these children is believed to be of viral origin, in which case antibiotic treatment would be unnecessary. (3;4) However, the differentiation between viral, bacterial or mixed infections is clinically impossible and bacteria can be cultured in a considerable proportion of patients.(5) Therefore most children ventilated with LRTI are treated empirically with antibiotics.(6;7) It is unclear whether the introduction of new rapid respiratory viral tests into the work-up of LRTI impacts antibiotic prescription.(8) The limited number of studies evaluating the effect of rapid diagnosis on antibiotic use yielded contradictory results.(9-14) The impact of viral testing in the PICU setting has not been investigated previously.

Rapid viral testing in the hospital setting has traditionally been performed with direct immunofluorescence tests (DIF). Currently, molecular amplification tests such as real-time polymerase chain reaction (PCR) have become the new golden standard for the detection of respiratory viruses because of their increased sensitivity.(15;16) Real-time PCR assays have been shown to be quick and reliable in the PICU population, leading to a large increase in diagnostic yield of respiratory viruses.(17-20) At present, the high costs of PCR assays are considered a disadvantage.

The aim of our study was to evaluate whether a viral diagnosis obtained by the more sensitive real-time PCR as compared to DIF, would impact antibiotic prescription in children admitted with LRTI at the PICU. To answer this question we first performed a multicenter survey study among PICU physicians. Subsequently, we conducted a 2-year prospective study at our PICU.

MATERIALS AND METHODS

Multicenter survey study

Respondents

A total of six PICU's from three large Dutch medical centers (University Medical Center Utrecht, Amsterdam Medical Center, Erasmus Medical Center Rotterdam) and three large German medical centers (University Medical Center Freiburg, Universitätsklinikum Mainz, Charité Berlin) participated in this multicenter study. A senior pediatric intensivist of each PICU listed the PICU physicians involved in the care of infants with LRTI at their PICU. Physicians were individually approached by the investigator or the pediatric intensivist for a response.

Questionnaire

Questionnaires consisted of five case descriptions, constructed from clinical data from five infants with typical signs of LRTI ventilated at our PICU in 2004. The infants were selected because they had a negative or indeterminate DIF test and a positive real-time PCR result. Characteristics of the five cases are shown in Supplementary table 1. After each case description the following question was asked: "Would you start/continue antibiotic therapy for this child?". Questions were first asked with the real-time PCR result concealed, and the same questions were repeated after disclosing the real-time PCR result. Anonymity of the respondents was assured. The questionnaire was pilot-tested to assure clarity and coherence, and it was subsequently modified in response to the pilot test results. The order of cases was random in the different questionnaires. Due to the low burden of the survey, ethical approval/informed consent was not needed according to the institutional review board of our hospital.

Prospective study

Patients

Ventilated patients under 5 years old admitted with LRTI to the PICU of the Wilhelmina Children's Hospital, who had a DIF test performed as part of routine investigation, were enrolled from October 2006 through October 2008. The Wilhelmina Children's Hospital is a tertiary care medical center with a 16 bed PICU facility. It serves as a referral center for the central part of the Netherlands. Exclusion criteria were asthma exacerbation, immune compromised state, indication for antibiotics other than LRTI, and repeated PICU admission for LRTI during the study period. Baseline clinical data were obtained from medical charts using standardized forms (gender, age, symptom onset, disease severity, antibiotics before admission, preterm birth, underlying illness, duration of ventilation, length of PICU stay, white blood count and C-reactive protein). Underlying illness was defined as chronic pulmonary disease, congenital heart disease, neurological disease or gastrointestinal disease.⁽²¹⁾ For the assessment of illness severity the oxygenation index (OI) was calculated 12 hours after admission ($OI = \text{fraction of inspired oxygen} \times \text{mean airway pressure} \times 100 / \text{partial oxygen pressure}$).

Viral testing

Nasopharyngeal aspirates were taken by trained nursing staff the morning of the first working-day after admission (working-days: Monday through Friday). Specimens were examined with DIF and real-time PCR as previously described.^(18;22-24) In short, part of the sample was subjected to DIF assays to detect respiratory syncytial virus (RSV), influenza viruses, parainfluenza viruses 1–3 and adenoviruses using Imagen kits (DaKo, Glostrup, Denmark), in accordance with the manufacturer's recommended protocol. Indeterminate DIF

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3 results were considered negative for the analyses. The remaining material was used for real-
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5 time PCR testing for RSV, influenza viruses, parainfluenza viruses 1-4, adenoviruses,
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7 rhinoviruses, coronaviruses, human metapneumovirus, *Mycoplasma pneumoniae*. During the
8
9 second year the newly detected human bocavirus was added to the panel.(25;26) Briefly, after
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11 nucleic acid extraction using the MagNA pure LC nucleic acid isolation system (Roche
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13 Diagnostics, Basel, Switzerland) amplification was carried out in a 25- μ l reaction mixture on
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15 a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The positive
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17 control for human bocavirus was provided by T. Allander, Karolinska Institute, Stockholm,
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19 Sweden. Internal control viruses were used to monitor for efficient extraction and
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21 amplification.
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29 Patient management **questionnaire**

30 Patient care of enrolled patients was left at the discretion of the attending PICU physician.
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32 Results of the DIF assays had a turn-around time of approximately five hours, and positive
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34 results were reported at the end of the first working-day to the attending clinician. Results of
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36 the real-time PCR assays had a turn-around time between 16 and 24 hours and the results
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38 were reported at the beginning of the second working-day to the investigator only. The
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40 investigator approached the attending physician before revealing the PCR result, using a
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42 questionnaire with the following questions: (1) “What is the patients’ suspected clinical
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44 diagnosis?” (To be answered with checkboxes for viral bronchiolitis, viral pneumonia, and
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46 bacterial pneumonia), and (2) “Is the patient treated with antibiotics?”. Subsequently, the
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48 physician was provided one hour later with the PCR result and the questions were repeated.
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50 Finally, PICU physicians were asked what the reason was for (dis)continuing antibiotics.
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52 Since the prospective study did not involve a change in patient management, ethical approval
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3 and/or informed consent of parents/guardian of infants was not required according to the
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5 institutional review board of our hospital.
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10 Statistical analysis

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12 For statistical analysis of the percentage of antibiotic use before and after disclosure of the
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14 real-time PCR results the McNemar test was used.
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RESULTS

Multicenter survey study

Respondents

Ninety-four PICU physicians (20 from Utrecht, 12 from Amsterdam, 16 from Rotterdam, 17 from Freiburg, 12 from Mainz, and 17 from Berlin) completed the questionnaire.

Antibiotic use

Before disclosure of the real-time PCR result, the percentage of PICU physicians that would treat the five infants with antibiotics was 37%-98% (range for the five cases; figure 1). After disclosure of the real-time PCR result the percentage of physicians that would treat the infants with antibiotics decreased to 19%-82% (McNemar test: $p < 0.001$ for all cases). The absolute reduction was largest for case 1 and 5 (37% and 42%). Although the percentage of antibiotic use pre-PCR test differed between centers, the reduction of antibiotic use following PCR was similar in the different centers.

Prospective study

Patients

Thirty-eight children with LRTI were enrolled in this study. Twenty (53%) children were clinically suspected of viral bronchiolitis, 9 (24%) of viral pneumonia and 9 (24%) of bacterial pneumonia. Demographics and clinical characteristics of the enrolled patients are provided in table 1.

Viral test results

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3 Nineteen patients were positive with DIF (50%), all for RSV; no (para)influenza or
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5 adenoviruses were detected (figure 2). Real-time PCR confirmed all 19 positive DIF results
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7 and detected 7 additional viruses in 6 of them (table 2). The remaining 19 patients were DIF
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9 negative. In 16 of the 19 DIF negative patients real-time PCR detected a total of 20 viruses. In
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11 3 (8%) patients with LRTI no virus could be identified either by DIF or by PCR. Therefore,
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13 an infection with one or more viruses was detected in 35 of 38 patients (92%).
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20 Antibiotic use before and after the PCR result

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22 In the group of 19 DIF positive patients, 9 (47%) were treated with antibiotics before the real-
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24 time PCR result was made available. Antibiotic prescription did not change after revealing the
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26 concordant positive real-time PCR results (figure 2).
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29 In the group of 19 DIF negative patients, 12 (63%) patients were treated with antibiotics
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31 before the PCR result was made available. The real-time PCR was positive in 9 of these 12
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33 patients. In none of the 9 patients the positive real-time PCR result prompted the PICU
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35 physicians to discontinue antibiotics. In only 1 of these 9 patients the suspected clinical
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37 diagnosis was changed by the attending physician from bacterial to viral pneumonia,
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39 nevertheless antibiotic treatment was continued. When asked for the reason to continue
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41 antibiotics, physicians listed (a) clinical suspicion of bacterial superinfection (ill-appearance,
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43 high ventilator settings, high C-reactive protein levels; n=7) and (b) waiting for the final result
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45 of the bacterial cultures (n=2). Finally, in the 7 DIF negative patients that were not treated
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47 with antibiotics, a negative real-time PCR result could have prompted physicians to start
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49 antibiotics. However, all 7 patients were found to be positive by real-time PCR for respiratory
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51 viruses; i.e. real time PCR confirmed the clinical suspicion of a viral infection and no
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53 antibiotics were started.
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3 In summary, 21 (55%) of the included 38 patients were treated with antibiotics and this
4 number was not influenced by the real-time PCR results. Of the 29 patients that were believed
5 by the PICU physician to have a viral lower respiratory tract infection (viral bronchiolitis or
6 viral pneumonia), 12 (41%) had antibiotics prescribed.
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14 15 **DISCUSSION**

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17 The present study investigated the impact of respiratory virus real-time PCR, in addition to
18 DIF, on antibiotic prescription in ventilated children with LRTI at the PICU, first in a survey
19 study and then in a prospective study. Although physicians indicated that real-time PCR
20 would have an effect on antibiotic use in the survey study, this effect was not found in
21 practice in the prospective study.
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31 A variety of viral tests are currently used in the diagnostic work-up of children with LRTI
32 at the PICU. Conventional rapid viral tests such as antigen detection by DIF are now being
33 replaced by more sensitive, molecular assays such as real-time PCR. This has led to an
34 increase in the detection of viruses,(18;27-29) with a substantial increase in costs.(22) To
35 determine if real time PCR theoretically could influence daily practice at the PICU, first a
36 multicenter survey study was performed. Since the survey study indicated that the availability
37 of PCR result would reduce antibiotic use, a 2-years prospective study was undertaken at a
38 single center to test the impact of real-time PCR in real-life.
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53 **Three** limitations of our study deserve further discussion. First, the number of evaluated PICU
54 children in the prospective study was relatively small. However, real-time PCR did not
55 influence antibiotic prescription in one single patient. Therefore, we believe that the inclusion
56 of additional patients is not likely to influence our observation that the impact of real-time
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3 PCR is minor. Second, the fact that physicians were aware of the study may have influenced
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5 their decision on antibiotic prescription. However, this would probably have led to an
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7 overestimation of the effect of real-time PCR results on antibiotic use which would only make
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9 the conclusion of our study stronger. Third, the turn around time of real-time PCR in our
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11 study was between 16 and 24 hours and PCR was performed on working-days, so the results
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13 were available only at the second working-day. Possibly, if results had been available earlier
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15 this could have had more effect on antibiotic prescription practices (physicians will probably
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17 be more reluctant to stop antibiotic treatment than not to start it). However, we aimed to
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19 evaluate the impact of real-time PCR in daily practice, and in most hospitals both DIF and
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21 real-time PCR are only performed on working-days (i.e. Monday-Friday) with the same turn
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23 around times for both tests.(30)
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32 In agreement with previous studies we showed that real-time PCR increased the diagnostic
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34 yield considerably when compared to DIF. The proportion of virus-infected children
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36 increased from 50% (DIF) to 92% (real-time PCR), and the number of viruses from a total of
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38 19 (all RSV) to 46 (8 different types). This increased diagnostic yield was not, however,
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40 accompanied by a decrease in antibiotic prescription. This finding is in contrast with our
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42 survey study in which real-time PCR decreased the percentage of antibiotic description by 16-
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44 42% (absolute reduction; $p < 0.001$ for all cases). Apparently, the treatment of paper patients is
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46 not the same as treating patients in daily PICU practice, in which physicians seem to prefer to
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48 stay on the safe side. In daily practice, physicians seem to make clinical decisions on other
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50 grounds than results from diagnostic investigations. Reasons for not stopping antibiotic
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52 treatment in PCR positive patients were the clinical suspicion of a (secondary) bacterial super
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54 infection, and the fact that the results of bacterial cultures were still unknown. Additional to
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56 the lack of effect of real-time PCR on antibiotic use found in this study, one may wonder what
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3 the effect of any viral test such as DIF may be, since a considerable proportion of patients
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5 (47%) received antibiotics regardless of a positive DIF result.
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10 Previous studies on the impact of rapid viral testing on antibiotic use in children have been
11 published. Our results are in agreement with Doan et al and Poehling et al, who did not find a
12 reduction in antibiotic prescription in children randomized to have rapid testing performed at
13 the emergency department.(10;12) Our results are in contrast with studies from Bonner et al,
14 Noyola et al, Sharma et al, and Woo et al, who found that physicians prescribed less
15 antibiotics in patient groups tested with a rapid viral test.(9;11;13;14) These studies were all
16 performed at the emergency department or at pediatric wards, and not at the PICU, and
17 assessed different age groups. It can be expected that physicians in a PICU setting are more
18 reluctant to withhold antibiotic treatment in the severely ill population with LRTI who
19 frequently has underlying diseases. This is reflected in our population by the finding that in
20 the 38 ventilated LTRI patients 21 children started with antibiotics at admission (55%) and
21 antibiotic prescription was not changed in any of the groups, not even in the 9 children with a
22 positive PCR and negative DIF.
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43 In addition, virtually all LRTI patients at our PICU (35 out of 38; 92%) were found to be
44 positive by real-time PCR for one or more viruses. This means that in our PICU population
45 virus-positivity does not help clinicians anymore to differentiate between those patients who
46 qualify for antibiotics from those who could safely be treated without antibiotics. Therefore,
47 we propose that future real-time PCR studies should be directed to settings where not all
48 patients are found to harbour viruses, such as the population of children with less severe
49 respiratory tract infection in the community.(10)
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3 Limiting antibiotic prescriptions in patients with viral LRTI is not the only objective of
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5 performing respiratory real-time PCR assays. Real-time PCR is valuable as a very sensitive
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7 tool to further explore the clinical presentation and impact of respiratory viruses as well as
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9 their epidemiology and it will become an even more important diagnostic tool when antiviral
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11 treatments become available. However, when real-time PCR in LRTI patients at the PICU
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13 does not affect daily practice, this test could be left out of the diagnostic work-up and costs
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15 could be saved.
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22 In conclusion, in daily practice real time PCR, in addition to DIF testing, has no impact on the
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24 prescription of antibiotics in ventilated LRTI patients at the PICU. This is in contrast to the
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26 response of physicians on paper case patients in a theoretical survey. This study shows a
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28 discrepancy between theoretical considerations and daily practice at the pediatric intensive
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30 care. In daily practice physicians seemed reluctant to stop antibiotic prescriptions in the
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32 severely ill population of LRTI patients at the PICU.
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3 **Figure legends**
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8 **Figure 1**
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10 Title: Antibiotic use before and after disclosure of the real-time PCR results in the survey
11 study
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14 Subscript: * Difference McNemar test $p < 0.001$
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19 **Figure 2**
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21 Title: Flow-chart of viral test results and antibiotic use of patients enrolled in the prospective
22 study.
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26 Subscript:
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29 ^aTwelve patients that were negative by direct immunofluorescence were treated with
30 antibiotics.
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34 ^bNine out of twelve patients that were negative by direct immunofluorescence and treated
35 with antibiotics were subsequently positive by real-time PCR. These are the patients in which
36 antibiotic prescription is most likely to be changed.
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Table 1

Demographics and clinical characteristics of patients enrolled in the prospective real-life study

Characteristics on admission [n=38]	Value
Sex, no. of males (%)	18 (50)
Age in month, median (IQR)	1.6 (8.8)
Symptom onset; median (IQR) no. days	3.0 (3.0)
Oxygenation index (OI) ^a	5.6 (2.8)
Antibiotics before admission, no. (%)	23 (61)
Preterm birth, no. of patients born <37 weeks (%)	9 (24)
Underlying Illness, no. (%)	
Pulmonary	9 (24)
Cardiac	2 (5)
Central nervous system	2 (5)
Gastrointestinal	1 (2.6)
Duration of ventilation in days, median (IQR)	9.0 (5.0)
Length of PICU stay in days, median (IQR)	9.5 (6.0)
White blood count, median (IQR) ^b	8.2 (3.8)
C-reactive protein mg/L, median (IQR) ^b	27.5 (49.0)

Abbreviations: IQR: interquartile range; PICU: pediatric intensive care unit; OI: oxygenation index

^aOxygenation index 12hr after admission; available for 23 patients with an arterial oxygen pressure measured at that time

^bFirst value after admission

Table 2

Viruses detected by real-time PCR in direct immunofluorescence [DIF] positive and negative children

Direct immunofluorescence positive patients (n=19) ^{ab}	No. patients (%)	Direct immunofluorescence negative patients (n=19) ^a	No. patients (%)
<i>Virus(es) detected by real-time PCR^c</i>		<i>Virus(es) detected by real-time PCR^c</i>	
Respiratory syncytial virus	13 (68)	Respiratory syncytial virus	4 (21)
Respiratory syncytial virus + rhinovirus	5 (26)	Influenza viruses	3 (16)
Respiratory syncytial virus + rhinovirus + coronavirus	1 (5)	Rhinovirus	1 (5)
		Coronavirus	1 (5)
		Adenovirus	1 (5)
		Human metapneumovirus	1 (5)
		Human bocavirus	1 (5)
		Respiratory syncytial virus + rhinovirus	1 (5)
		Respiratory syncytial virus + human bocavirus	1 (5)
		Respiratory syncytial virus + human metapneumovirus	1 (5)
		Coronavirus + parainfluenza virus	1 (5)
Total no. of real-time PCR positive patients^d	19 (100)	Total no. of real-time PCR positive patients^e	16 (84)

^aDirect immunofluorescence [DIF] was performed for respiratory syncytial virus, influenza viruses, parainfluenza viruses and adenoviruses.

^b The DIF positive patients were all positive for respiratory syncytial virus .

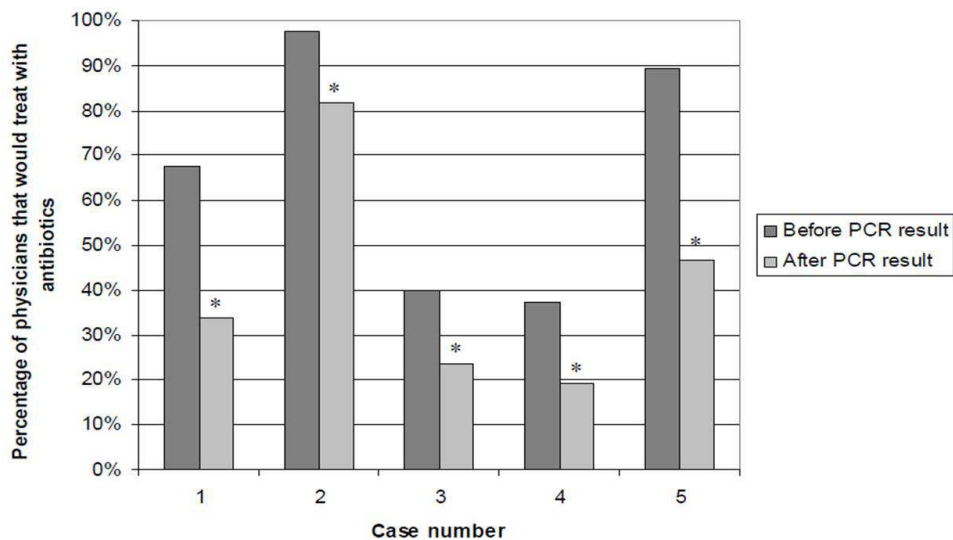
^cPCR was performed for respiratory syncytial virus, influenza viruses, parainfluenza viruses, adenoviruses, rhinoviruses, coronaviruses, human metapneumovirus, *Mycoplasma pneumoniae*, and human bocavirus.

^dIn the DIF positive group real-time PCR identified a total of 26 viruses (The 19 viruses identified by DIF plus 7 additional viruses).

^e In the DIF negative group real-time PCR identified a total of 20 viruses.

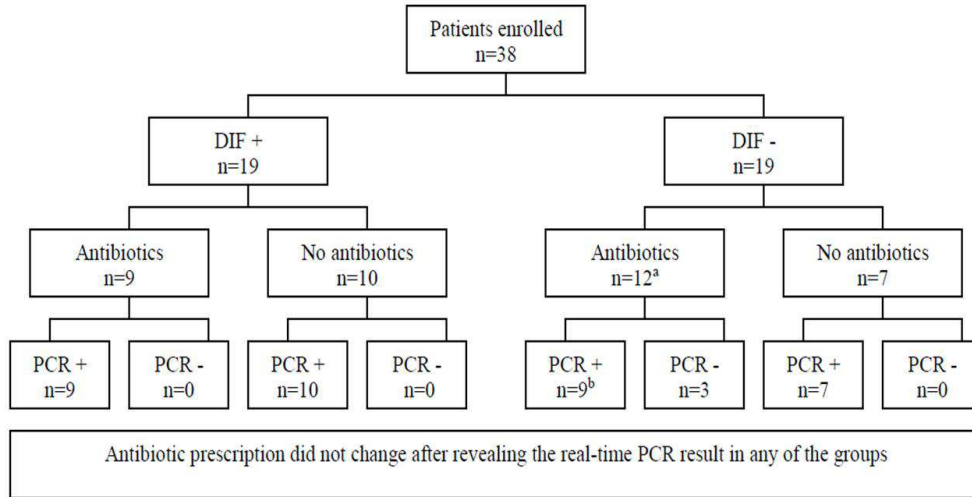
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Figure 1



271x169mm (120 x 120 DPI)

Figure 2



261x163mm (125 x 125 DPI)

Review

Supplementary table 1 Clinical characteristics of the five cases in the questionnaire of the paper case study

	Case 1	Case 2	Case 3	Case 4	Case 5
Age	2.5 months	3 months	6 weeks	8 months	3 weeks
History	Previously healthy	Dysmature	Spina bifida	Wheezer	Previously healthy
Auscultation	Rales	Rales/Creptitations/ Wheeze	Normal	Creptitation/Wheeze	Normal
Chest X-ray	Possible infiltrate	Infiltrate	Hyperinflation	Hyperinflation	Opacity
C-reactive protein	<0.7 mg/dL	9 mg/dL	<0.7 mg/dL	1.1 mg/dL	6.0 mg/dL
WBC	Not assessed	5.6*10 ⁹ /L	11*10 ⁹ /L	10.6*10 ⁹ /L	10.2*10 ⁹ /L
Antibiotics ^a	Yes	Yes	No	No	No
DIF	Negative	Negative	Indeterminate	Indeterminate	Negative
PCR	Parainfluenza virus, Rhinovirus	RSV, Coronavirus	RSV, Rhinovirus	RSV, Adeno, Rhino, and Coronavirus	RSV

Abbreviations: DIF: direct immunofluorescence; PCR: polymerase chain reaction, respectively; WBC: white blood cell count; RSV: respiratory syncytial virus.

^a antibiotics initiated before admission (in the real case)