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Low prevalence of human metapneumovirus and human bocavirus in adult immunocompromised high risk patients suspected to suffer from Pneumocystis pneumonia

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Summary  Background: Novel respiratory viruses were discovered in the last years predominantly in children. Until now information on newly identified respiratory viruses in immunosuppressed adult patients is limited. Here we investigated immunocompromised adults with suspected Pneumocystis jirovecii pneumonia (PCP) for new respiratory viruses.

Methods: Bronchoalveolar lavage (BAL) samples of 128 patients with atypical pneumonia (HIV-infected n = 50, hematological malignancy n = 51, immunosuppressive treatment n = 27) were prospectively evaluated for P. jirovecii and retrospectively for new respiratory viruses (HMPV, HBoV, HCoV-NL63/SARS/HKU1).

Results: P. jirovecii was detected in 26/128, bacteria in 10, fungi in four, Influenza A in two patients. Novel respiratory viruses were found in only two/128 patients with hematological malignancy, of those one patient with HBoV-infection and one with HMPV-infection, respectively. No pathogens were detected in 82/128 patients. The one patient with detection of hMPV and clinical diagnosis of atypical pneumonia died of pulmonary failure.

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Conclusion: Human bocavirus and human metapneumovirus are rarely involved in atypical pneumonia in immunocompromised adult patients with suspected PCP, but may contribute to severe respiratory failure.

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Introduction

Besides the commonly known viruses that cause respiratory tract infections like respiratory syncytial virus (RSV), rhinoviruses, adenoviruses, and the human coronaviruses (OC43 and 229E) an increasing number of “new” respiratory viruses were detected in the last years. These viruses include the human metapneumovirus (hMPV) and the human bocavirus (hBoV) first described in 2001 and 2005,1,2 as well as the human coronaviruses NL63 and HKU1,3,4 respectively. Most epidemiological and clinical studies have focused on children, where hMPV is associated for up to 25% of viral respiratory tract infections.2,5 Unlike hBoV, the relationship between hMPV and symptomatic respiratory tract infection is undoubted. Since the first description of hBoV an increasing number of prevalence studies were published but until now disease association in children with acute respiratory tract infections remain unclear. So far it was controversially discussed whether human bocavirus is indeed a pathogen rather than an innocent bystander as it is frequently associated with coinfections and Koch’s modified postulates cannot been fulfilled.6 Data for adults especially in immunocompromised patient are limited for both viruses.7,8

We studied retrospectively bronchoalveolar lavage specimens for human bocavirus and human metapneumovirus from immunocompromised adult patients with different underlying disease and atypical pneumonia who were suspected for Pneumocystis jirovecii pneumonia (PCP).

Patients and methods

Bronchoalveolar lavages from 157 immunocompromised patients with clinical and radiological confirmed atypical pneumonia were sent to the Department of Infectious Disease at the University of Cologne during a 2 year period between January 2003 and December 2004. Samples were aliquoted for preparing DNA/RNA, for light microscopy and storing at –80 °C.

All BAL samples were prospective examined for Pneumocystis jirovecii, Mycobacterium tuberculosis, Chlamydia pneumoniae, Aspergillus sp., Candida sp., Cryptococcus neoformans, cytomegalovirus, Ebstein–Barr virus, Herpes simplex and Varicella zoster, Influenza viruses A and B by culture, antigen detection, light microscopy, PCR or RT PCR, as appropriate.9

BAL specimens for examination of P. jirovecii by light microscopy were concentrated by a modified water—ether sedimentation procedure. A part (20 μl) of the suspension was used for preparing smears (about 15 mm²). Smears were fixed in methanol and stained with Uvitex 2B (Fungigual A, R&R, Kandern, Germany), and examined at ×400 as well as ×1000 magnification under Zeiss fluorescence microscope (Oberkochen, Germany) as previously described.10

DNA and RNA extractions for PCR detection were prepared from BAL by using a QI Amp Tissue Kit and the viral RNA kit (Qiagen, Hilden, Germany), respectively, following the manufacturer's instructions. PCR amplifications were done in 50 μl reaction mixtures under the following conditions: 25 pmol of each primer, 200 μM each of deoxynucleoside triphosphate, 10 mM Tris–HCL (pH 9.0), 50 mM KCL, 1.5 mM MgCl2, and 2.5 U of Taq DNA polymerase (Perkin–Elmer, Norwalk, CT, USA). Reactions were run in a Perkin–Elmer thermocycler using a step cycle programme. After initial denaturation of DNA at 94 °C for 3 min, 35 cycles were run: 94 °C for 1 min, annealing temperature of 55 °C for 2 min, and 72 °C for 3 min with a 10 min 72 °C extension after the 35 cycles.11–14 Primers pAZ102-H (5’-GTG TAC GTT GCA AAG TAC TC-3’) and pAZ102-E 5’-GAT GCC TGT TTC CAA GCC CA-3’ were used to amplify a 346 base-pair DNA fragment of the mitochondrial LSU-rRNA gene of P. jirovecii.15

Retrospectively, RSV, hMPV, coronaviruses NL63, HKU1, OC43, and 229E, and the human bocavirus (hBoV) were detected PCR or RT PCR, as previously described.15 Sensitivities for RT-PCR reactions for RSV, NL63, and hMPV were 1 × 10-5–5 × 10-4 genome equivalents per ml (as determined with infected cell culture supernatants) and 10-104 genome equivalents per ml clinical samples for human bocavirus as determined with plasmid DNA.

A 10 μl aliquot from each reaction was run on a 3% Nu-Sieve 3:1 electrophoresis-grade agarose gel (FMC Bioproducts, Rockland, Maine, USA) in 1× TAE buffer (0.04 mol/l Tris acetate, 0.001 mol/l EDTA) with ethidium bromide (0.5 g/ml) to visualize the amplified PCR products under UV-illumination.

Procedures for avoiding contamination were strictly followed. DNA/RNA isolation, preparation of reaction mixtures, and amplification and analysis were physically separated and performed in three different rooms. Positive displacement tips were used for all manipulation and negative controls containing reaction mixtures without DNA were always done.

Results

BAL samples from 157 immunocompromised patients were examined for detection of P. jirovecii. Because of insufficient amounts of BAL samples 29 of these patients were excluded from retrospective laboratory analysis for new or emerging viruses.

Of 128 patients included in the analysis 50 (39.1%) patients were HIV-infected, 51 (39.8%) suffered from hematological malignancy (e.g. leukemia, lymphoma) and 27 (21.1%) were treated with immunosuppressive medication because of solid organ transplantation (e.g. kidney, heart) or other multisystemic diseases.

Overall P. jirovecii was detected in 26 of 128 (20.3%) patients included in the retrospective analysis: 17 of 50 (34%) HIV-infected patients, five of 51 (9.8%) patients with...
hematological malignancy and four of 27 (14.8%) patients with immunosuppressive treatment due to other causes (Table 1). Several bacteria were cultured in 10 of 128 (7.8%) patients and fungi were found in five of 128 (3.9%) patients.

Viruses were detected in five of 128 (3.9%) patients; CMV in one patient with hematological malignancy, Influenza A in two patients (one HIV-infected patient and one with immunosuppressive medication), RSV, Influenza B and coronavirus were not detected in any BAL specimens. HMPV was detected in a BAL specimen of a male patient with chronic myeloid leukemia and allogenic bone marrow transplantation. He was admitted with severe dyspnea, cough and fever and atypical pneumonia were diagnosed by chest X-ray and computed tomography. Bronchoscopy showed hyperemia of the tracheal mucosa suggesting inflammation and histological analysis of lung biopsies resulted in infiltrates with lymphocytes and plasma cells and the additional diagnosis of an adenocarcinoma of the lung. The patient did not show the "sepsis-like" syndrome or pulmonary hemorrhage previously described by Englund and coworkers. Unfortunately the patient died due to a severe aggravation of the atypical pneumonia and fatal pulmonary failure 10 days after admission.

Human bocavirus was detected in a BAL specimen of a female patient with lymphoma and induced immunosuppression due to chemotherapy. She developed a fever of unknown origin and was treated with antibiotics, antymycotic treatment and with ganciclovir because of suspected CMV infection. The patient reported ongoing pneumonialike symptoms and atypical pneumonia was diagnosed. A BAL specimen was taken and the only positive result was hBoV. Within a few days the symptoms improved continually and the patient was discharged from hospital.

Discussion

Pneumocystis pneumonia is one of the leading causes of disease and frequently the first severe AIDS defining illness in HIV-infected patients. Since the introduction of PCP prophylaxis and the initiation of high active antiviral therapy (HAART) the incidence of PCP declined from approximately 90–30 per 1.000 person years in HIV-infected patients. Despite this improvement, until now PCP is still the most common AIDS defining opportunistic infection in Northern America and Europe. PCP is also recognized in patients with malignancy, after solid organ transplantation and immunosuppressive treatment.13

In our high risk immunocompromised patients who underwent diagnostic procedure including BAL because of atypical pneumonia 20% had a diagnosis of PCP most of these patients were HIV-infected (group 1). However, in more than 60% of the examined patients no pathogen was detected and several other pathogens were found in only 20%. Newly described respiratory viruses were only detected in two patients consistent to previously published studies. Englund et al. evaluated retrospectively BAL specimens of adult patients after hematopoietic stem cell transplantation for the presence hMPV and detected in five of 163 (3%).7 Of these five patients, four died of respiratory complications like our patient at a median of 16 days. In contrast none of the eight adult patients with pneumonia, sole detection of hMPV and several underlying diseases died in a recently published study by Johnestone et al. but immunocompromised patients were excluded from the analysis.14 Immunocompromised patients seem to be apparently more severely affected than non-immunocompromised patients like it is observed for several other viruses. Barenfanger et al. tested 161

| Pathogen (%) | All (%) | Group 1 (%) | Group 2 (%) | Group 3 (%) |
|--------------|---------|-------------|-------------|-------------|
| Pneumocystis jirovecii | 26 (20.3) | 17 (34.0) | 5 (9.8) | 4 (14.8) |
| RSV | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| HBoV | 1 (0.8) | 0 (0.0) | 1 (2.0) | 0 (0.0) |
| HMPV | 1 (0.8) | 0 (0.0) | 1 (2.0) | 0 (0.0) |
| Influenza A | 2 (1.3) | 1 (2.0) | 0 (0.0) | 1 (3.7) |
| Influenza B | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Coronavirus | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| CMV | 1 (0.8) | 0 (0.0) | 1 (2.0) | 0 (0.0) |
| Bacteria | 10 (7.8) | 4 (8.0) | 5 (9.8) | 1 (3.7) |
| Fungi | 5 (3.9) | 0 (0.0) | 4 (7.8) | 1 (3.7) |
| Copathogen | 1 (0.8) | 1 (2.0) | 0 (0.0) | 0 (0.0) |
| No pathogen | 82 (64.1) | 29 (58.0) | 32 (62.7) | 21 (77.7) |
patients older that 20 years and 230 patients younger than 20 years and detected hMPV in 3.1% vs 2.6%. Notable was also that only one of the children but all of the adults were hospitalized.\textsuperscript{15} Kamboj and coworkers most recently found that HMPV-infections in cancer patients caused a number of unspecffic respiratory symptoms but observed no fatal cases of respiratory HMPV-infections\textsuperscript{16} in their cohort. Thus, the cases described here and by Englund et al. may have suffered from additional predisposing conditions that have not been identified so far. However, two further studies described that infection by HMPV was 5.8–9% in hematopoeitic cell transplant recipients, thus there my be indeed more severe but so far neglected cases in such cohorts.\textsuperscript{17,18}

The clinical relevance of hBoV is uncertain in contrast to hMPV. The proportion of respiratory specimens from symptomatic hospitalized children that contains hBoV DNA ranged between 1.5% and 19%,\textsuperscript{6,8,19–31} and as recently reviewed by our group.\textsuperscript{6} The high rate of co-infections with other viral pathogens suggests that the virus is a possible passenger. In an unpublished prospective matched-pair analysis our working group have shown that in hospitalized children hBoV-infection appears to be as serious as RSV-infection and these data demonstrated that human bocavirus is an independent and severe pathogen (Schildgen et al., submitted). For adult patients the importance of hBoV as a respiratory pathogen is unclear. Recently, Longtin et al. detected hBoV in only one of 126 (0.8%) symptomatic adults with bronchitis or pneumonia.\textsuperscript{8} However, we have recently described a clinical case of a severe pneumonia most likely associated to hBoV in an adult patient that was also confirmed by radiological findings.\textsuperscript{32} hBoV in immunocompromised pediatric patients were described after organ transplantation and in HIV-infected children.\textsuperscript{33,34} Occurrence of hBoV in adult immunocompromised patients as described in the presented study seems to be rare but prospective studies are lacking.

This study shows that not only young infants can be affected from infections with “new” respiratory viruses, e.g. hMPV or hBoV. These viral pathogens should be carefully included in routine testing in adult patients with atypical pneumonia but further studies are necessary to prove the clinical relevance in adults especially for hBoV.

Conflict of interest

None of the authors have any conflict of interest.

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Appendix A. Supplemental material

Supplementary information for this manuscript can be downloaded at doi: 10.1016/j.jinf.2009.01.004.

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