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an active infection of cardiovascular tissues by these viruses may contribute to the pathogenesis of coronary disease.

05 The role of respiratory viruses in developing bronchiolitis obliterans and IPS in pediatric HCT patients

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Background: Respiratory viruses (RVs) infections are associated with bronchiolitis obliterans (BO) and rejection in lung transplant patients and may progress to pneumonia or trigger immunological mediated effects on lung function in hematopoietic cell transplantation (HCT). We studied the clinical impact of RVs in pediatric HCT patients.

Methods: Weekly nasopharyngeal aspirates of patients were examined by real-time PCR for respiratory viruses. Initial clinical symptoms and long term complications were noted.

Results: 61 HCTs were done from 31 matched donors and 30 mismatched donors. Overall survival was 65%. In 23 patients a RV was identified. Initial clinical symptoms were usually mild. Most patients did not clear the virus for weeks to months. Idiopathic pulmonary syndrome (IPS) occurred in 7 patients within a median period of 8 weeks post HSCT. Despite treatment with pulsed high dose methylprednisolone (HDMP) 6 patients needed ventilation and 2 of them died. BO occurred in 4 patients within a median period of 4 months after HSCT. Pulsed HDMP was given, with only partial response. 2 patients died and 1 is awaiting lung transplantation. In the RV negative group only 1 patient developed severe pulmonary complications. In a multivariate analysis (possible confounders age, sex, HLA-disparity, source, GVHD) a RV-infection before or early after HCT was a significant risk factor for developing IPS/BO (p=0.005, RR 31, range 2.8–334).

Conclusion: RV infection early after HCT is associated with severe pulmonary complications and mortality. The exact role of RV in these complications needs further evaluation. Post-viral fibrosis due to long term viral persistence and/or triggered allo-reactivity may cause these complications.

06 Herpes simplex (HSV) viral load in bronchoalveolar lavage: risk factors and clinical outcome

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Introduction: Since the relevance of detecting HSV-1,2 in bronchoalveolar lavage fluid (BALF) is unclear, we studied the correlation of HSV-1,2 viral load in BAL with clinical variables and outcome.

Materials and Method: 520 BAL fluid samples were collected from 382 intensive care unit (ICU) and 138 non-ICU patients suspected of pneumonia and analyzed by quantitative real time polymerase chain reaction (PCR). Immunofluorescent staining (IF) of cytospin preparations was performed of PCR positive samples.

Results: HSV-1 DNA was detected in 27% of ICU-patients and 7% of non-ICU patients. In the age group <50 years HSV-1 DNA was less frequently isolated compared to patients ≥50 years (11% and 28% respectively, p<0.001). HSV-1 was not correlated with gender, smoking, length of hospital-stay, SOFA-score and the concomitant presence of ventilator associated pneumonia. HSV-1 PCR >log5 genome equivalents/ml in BAL (35/520) was associated with increased 14 day-mortality (p-value = 0.018). HSV-2 was detected in low quantities in two non-ICU patients and was not associated with morbidity or mortality. HSV pneumonia was histologically proven in two patients (HSV-1 DNA >log5). IF staining was positive in samples containing HSV-1 in quantities >log7/ml.

Conclusions: Admittance to the ICU and age above 50 years were risk factors for HSV-1 recovery in the lower respiratory tract.

Detection of HSV-2 in BAL was rare. The higher mortality observed in patients with HSV-1 viral loads >log5/ml enforces its clinical relevance and the necessity to start randomized medical intervention studies.

07 Multiplex RT-PCR for detecting nineteen respiratory viruses

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Background: Virology laboratories traditionally use DFA and culture to diagnose respiratory virus infections. With the discovery of five new respiratory viruses since 2000 there is a need for tests to detect these additional agents. We have developed a test that can detect 19 respiratory viruses in a single test.

Methods: Total RNA and DNA was extracted from NP specimens, reverse transcribed into cDNA and amplified by multiplex PCR using 14 primer pairs. Amplimers were interrogated by a multiplexed Target Specific Primer Extension (TSPE) reaction using 21 primers specific for virus types/subtypes. TSPE products were labeled with biotin, addressed to individual microspheres using a tag anti-tag hybridization (TmBiosciences Corp'n) and signals detected using a Luminex-100 instrument.

Results: The assay detected the following viruses: Influenza A subtypes H1, H3, and H5 including the H5N1 Asian lineage virus, Influenza B, Parainfluenza types 1, 2, 3, and 4, RSV types A and B, Adenovirus, Metapneumovirus, Rhinovirus, Enterovirus, Coronaviruses OC43, 229E, SARS-CoV, NL63, and HKU1. In an evaluation of 294 NP specimens the test had a sensitivity of 98% (127/132) compared to DFA and culture. The test detected 30% more positive specimens (all confirmed by second PCR) including Rhinovirus, Enterovirus, or Coronaviruses not tested for by the clinical laboratory and 5.2% dual infections.

Conclusions: We have developed a new test that can detect 19 respiratory viruses in a single test including conventional viruses, common cold viruses (Rhinovirus and Coronaviruses) and newly emerging viruses (SARS and H5N1). This test should improve the viral diagnostic capability of hospital and public health laboratories and provide a tool for epidemiological studies.

08 Human bocavirus and acute wheezing in children

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Background: Human bocavirus is a newly discovered parvovirus. It has primarily been detected in children with acute respiratory tract infection, but its prevalence, clinical profile and role as a causative agent of respiratory tract disease is not clear.

Methods: We investigated the presence of human bocavirus by quantitative PCR in respiratory tract samples and selected serum samples of 259 patients hospitalized for acute expiratory wheezing. The samples were analyzed for 16 respiratory viruses by polymerase chain reaction, virus culture, antigen detection and serological assays.

Results: At least one potential etiologic agent was detected in 95% and more than one agent in 34% of cases. Human bocavirus was detected in 49 (19%) children. A large proportion of these were mixed infections with other viruses, but human bocavirus was the only virus detected in 12 (5%) cases. High bocavirus copy number infection was preferentially seen in the absence of other viral agents, supporting its causative role for acute wheezing. In addition, low copy number infection was prevalent. Nasal swabs from 64 asymptomatic control patients were negative for human bocavirus. Human bocavirus DNA was frequently detected in the serum of patients with acute wheezing, suggesting systemic infection.

Conclusions: Human bocavirus causes a systemic infection and is often associated with acute childhood wheezing. Human bocavirus is probably a common causative agent of acute respiratory infections in children.