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## Oral Presentations

### O1 Bioportfolio: lifelong tissue persistence of new and old parvoviruses

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**Background:** Human erythrovirus (B19) is a minute single-stranded DNA virus causing many diseases. Following primary infection, the viral genomes persist in solid tissues. Besides the prototype, virus type 1, two major variants (types 2 and 3) have been identified recently, the clinical significance and epidemiology of which are mostly unknown. In this work we have determined the extent and duration of persistence of genomic DNA of the different erythrovirus types in a large number of tissue samples and sera.

**Methods:** Samples of skin, synovium, tonsil or liver (n = 523, birth-year range 1913–2000), and 1640 sera, were examined by PCR for the DNA of human erythroviruses.

**Results:** Virus types 1 and 2 were found in 132 (25%) and 58 (11%) tissues, respectively. DNA of virus type 1 was found in all age groups, whilst that of type 2 was strictly confined to subjects born before 1973 (P < 0.001). Virus type 3 was undetectable in any of the tissues studied. The sera from the past two decades contained only DNA of type 1.

**Conclusions:** Erythrovirus types 1 and 2 circulated in Northern and Central Europe in equal frequency from the 1930s to the 1950s. However, type 2 have disappeared from wide circulation by the 1970s and remained absent thereafter. Type 3 never attained wide occurrence in this area during the past 70 years. Our data indicate that human tissues possess, regarding the genomes of single-stranded DNA viruses, a storage mechanism of life-long capacity. For this new concept we propose the term Bioportfolio.

### O2 Analysis of HPV16 E6\*–II transcripts in cervical samples

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In patients infected by high risk HPV integration of viral DNA and over expression of E6–E7 proteins play an important role in the development of cervical preneoplastic lesions and invasive cancer.

The aim of this study was to analyse the two spliced transcripts of HPV16 E6/E7 ORFs, termed E6\*<sup>I</sup> and E6\*<sup>II</sup>, and to correlate the expression pattern to the physical status in a series of HPV 16 DNA positive patients with abnormal Pap smears. The detection of E6\*<sup>I</sup> and E6\*<sup>II</sup> was performed by qRT-PCR using intron/exon boundary spanning primers. The detection of physical status was performed by qPCR assay for E2 and E6 genes. A total of 41 cytological specimens were collected from not treated patients with diagnosis of LSIL (N = 19) and HSIL (n = 22).

The expression of both E6\* was absent in 27% of LSIL, whereas none of the HSIL was negative. The only expression of E6\*<sup>II</sup> was present exclusively in LSIL (42%), whereas the only expression of E6\*<sup>I</sup> (18.18%) was present exclusively in HSIL. The expression of both E6\* was present in 32% of LSIL and in 81.82% of HSIL, moreover the ratio E6\*<sup>I</sup>/E6\*<sup>II</sup> is > 1 in 17% of LSIL and in 94% of HSIL. The expression of only E6\*<sup>I</sup> or of both transcripts was significantly associated to the presence of mixed or integrated DNA

forms. In conclusion, the over expression of both E6\*<sup>I</sup> and E6\*<sup>II</sup>, supported by the presence of mixed or integrated DNA, have a negative prognostic value for cervical disease progression.

### O3 Laboratory containment of poliovirus and potentially infected material after global eradication of poliomyelitis

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The World Health Organisation's (WHO) Global Plan for Laboratory Containment of Wild Polioviruses was published in 2004 [1] and describes two phases of activities leading to containment – the laboratory/inventory phase and the global certification phase. Although the likelihood of subsequent transmission of wild poliovirus from laboratory to community is considered to be small, the consequences in a post-eradication era would be serious. An update on the UK progress towards meeting the laboratory and inventory phase of the WHO global plan will be presented. This includes completion of a survey of laboratories holding poliovirus and potentially infected material; and commencement of laboratory audits, as part of Health & Safety Executive's routine inspection programme, which will be reported to the UK's National Containment Co-ordinator at the Health Protection Agency. Since the certification of the global eradication of poliovirus is somewhat off, the need for laboratories to maintain the currency of their inventory of poliovirus and potentially infectious material is paramount.

#### References

[1] <http://www.polioeradication.org/content/publications/WHO-VB-03-729.pdf>

### O4 Enterovirus cardiovascular infection and acute myocardial infarction

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**Background:** Enteroviruses, particularly coxsackievirus B, have been suspected to play a role in the development of acute myocardial infarction (MI).

**Aims:** In the present study, we evaluated the potential role of enterovirus cardiovascular infections in the pathogenesis of MI.

**Methods:** The presence of enterovirus 5'-non-coding sequences and VP1 capsid protein was retrospectively investigated by RT-PCR and immunohistochemistry assays in myocardial tissues of patients who died suddenly of acute MI between September 1998 and December 2003, by comparison to similar samples of control patients matched for gender, residence area and year of death. For each sample positive for enterovirus genomes or antigen detection, an enterovirus strain typing was performed by partial amplification, TA cloning and sequencing of the VP1 capsid gene.

**Results:** Enterovirus markers were detected in 20 (40%) of 50 patients who died suddenly of MI, 2 (4%) of 50 matched subjects without cardiac disease (P < 0.001) and 4 (8%) of 50 matched patients exhibiting a non-coronary chronic cardiopathy (P < 0.001). All of the enterovirus positive patients exhibited VP1 protein that provided evidence of viral myocardial replication activity. VP1 gene sequences amplified after cloning from myocardial samples of 8 of the MI patients showed a strong homology with sequences of coxsackievirus B2 and B3 serotypes.

**Conclusions:** The demonstration of enteroviral replication activity and the identification of coxsackievirus B sequences in the heart samples of several patients that died suddenly of MI, suggests that

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.