P501
Bacterial colonisation/infection and airway inflammation in preschool children with recurrent wheeze
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Background: Wheeze is a common symptom in preschool children. The role of bacteria in preschool wheeze and their possible association with airway inflammation is largely unknown.

Aims:
- To study whether preschool children with recurrent wheeze differ in bacterial infection/colonisation in the upper respiratory tract compared with healthy controls.
- To study whether recurrent wheezers with bacterial infection/colonisation differ in pro-inflammatory markers in exhaled breath condensate (EBC) compared with infected/colonised children without recurrent wheeze.

Methods: We recruited 252 children (aged 2-4 years) with (n=202) and without (n=50) recurrent wheeze. Nasal and throat swabs were analysed for the presence of Streptococcus (S.) pneumoniae and Haemophilus (H.) influenzae. Serology for Chlamydia (C.) and Mycoplasma (M.) pneumoniae was assessed. EBC was collected using a closed-glass condenser. Immunoassay for H. influenzae (Interleukin (IL)4, IL-8, IL-13) in EBC were measured using multiplex immunoassay.

Results: Only positive serology for M. pneumonia was slightly higher in children with recurrent wheeze compared with healthy controls (11% vs. 2%, p=0.09). Wheezing children colonised with H. influenzae (N=61) had higher levels of all interleukins compared with colonised children without recurrent wheeze (N=15) (p<0.05).

Conclusion: We found no convincing evidence for an association between bacterial colonisation/infection and preschool recurrent wheeze. Airway colonisation with H. influenzae leads to elevated pro-inflammatory markers in recurrent wheezers, indicating augmented airway inflammation in these children.

P502
Small airway function in prematurely born infants following viral infection
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Aim: Respiratory syncytial virus (RSV) lower respiratory tract infections (LR-TIs) in prematurely born infants have been associated with abnormalities of lung function at follow up, and there is some preliminary evidence to suggest other viral infections may have a similar adverse effect. The aim of this study was to determine whether lung clearance index (LCI), an assessment of small airway function, differed between infants who had had an RSV LRTI, another viral LRTI or no LRTI.

Methods: 78 infants born at less than 36 weeks gestational age were prospectively followed. A nasopharyngeal aspirate (NPA) was obtained every time the infants had a LRTI regardless of whether this was in hospital or in the community. NPAs were tested for RSV A and B, Rhinovirus, human Metapneumovirus, Parainfluenza 1-3, Influenza A and B and Adenovirus. LCI was measured by the multiple breath wash-out technique at one year corrected age. Recruitment of at least 12 infants into each group allowed us to detect a difference in the LCI results of 0.7 between the groups (80% power, 5% level).

Results: Seventy two infants had acceptable measurements: 13 infants developed RSV LRTIs (RSV group), 17 infants other viral LRTIs (other viral group) and 34 infants no LRTI (no LRTI group). There were no significant differences in the LCI results of the three groups (RSV group mean [SD] 7.24 [0.44]; other viral group 7.0 [0.64]; no LRTI group 7.25 [0.63]) (p=0.69).

Conclusion: These results suggest viral infections are not associated with increased small airway abnormalities at follow up during infancy of prematurely born infants.

P503
IL-877 isoform in lungs of preterm infants and its processing by neutrophil serine proteases
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Introduction: Persistent neutrophilic (PMN) lung inflammation is strongly implicated in the development of Chronic Lung Disease (CLD) of Prematurity. The 72 amino acid (a.a.) chemokine, IL-872, is a key molecule involved in attracting PMNs to sites of inflammation. The longer 77 a.a. isoform (IL-877) is less potent than IL-872 in vitro. We studied expression of IL-877 in the preterm ventilated lung and its processing by neutrophil serine proteases.

Methods: IL-877 was measured by ELISA in bronchoalveolar lavage fluid (BALF) from ventilated preterm infants born at ≤32 weeks gestational age and after conversion by purified PMN serine proteases. Results were compared between the CLD (persistent inflammation) and non-CLD groups (resolved).

76. Innate and exogenous factors in childhood respiratory infection

PS01
Bacterial colonisation/infection and airway inflammation in preschool children with recurrent wheeze
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Results: The majority of IL-8 consisted of the shorter isoforms in the preterm lung. The IL-8 isoforms were expressed well with total IL-8 (p < 0.0001) but not with gestation (p = ns). Stained adult airway cells and neonatal PMNs and monocytes expressed IL-8 in the preterm 18 h (p<0.05), which was inhibited by α-1 antitrypsin (AAT). Purified neutrophil serine proteases converted IL-8 to shorter isoforms dose-dependently and over time.

Conclusions: Majority of IL-8 in the ventilated preterm lung are the potenter shorter isoforms. Although potentially expressed in the lung, IL-8 was probably converted to the shorter isoforms by neutrophil serine proteases. Inhibition of conversion by AAT suggests a potential therapeutic role for it in modulating inflammation in the preterm lung.

P504
Expression and functional activity of IL-6, sIL-6R & sgp130 in the preterm infant lung
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Introduction: Persistent neutrophil-dominated lung inflammation is strongly implicated in the development of Chronic Lung Disease of Prematurity (CLD). The complex of interleukin-6 (IL-6) and its soluble receptor, sIL-6R, can activate trans-signalling in cells lacking the cell-surface receptor IL-6R; a soluble form of the signal-transducer gp130 can inhibit trans-signalling by specifically binding to the sIL-6R/L-6 complex. As anti-inflammatory effects of IL-6 trans-signalling can shape resolution of inflammatory responses, we studied the expression, interactions and functional activity of IL-6, sIL-6R and sgp130 in the preterm lung.

Methods: Cytokines were measured by ELISA in bronchoalveolar lavage fluid (BALF) of ventilated preterm infants born at <32 weeks gestational age. Functional activity was determined in a sensitive, IL-6 specific bioassay using mouse B9 cells. Results were compared between the CLD group (persistent inflammation) and control (non-CLD) group (resolved).

Results: Inflammatory cells and chemokines, CXCL8 and CCL2, were higher in the CLD group (p<0.05). IL-6 and sIL-6R were comparable between the two groups. Baseline IL-6 levels in the CLD group (CLD 65.3 ng/ml vs. non CLD 20.8 ng/ml p=0.01), as was the anti-trans-signalling ratio of sgp130/IL-6R (p=0.02). Functional activity of IL-6 in the bioassay was similar between the two groups. Trans-signalling activity was not noted in any of the samples in the bioassay.

Conclusions: Increased sgp130 in the lungs of preterm infants may be responsible for impaired trans-signalling by the sIL-6R/L-6 complex. Better understanding of this pathway may lead to therapies to resolve lung inflammation in preterm infants.

P505
Cytokine response in pediatric patients with pandemic H1N1 influenza virus infection and pneumonia: Comparison with pediatric pneumonia without H1N1 infection
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Objectives: We investigated serum cytokine levels in pediatric patients with pandemic H1N1 virus infection and pneumonia but without H1N1 infection, and examined correlations between cytokine levels and clinical/laboratory findings.

Methods: Fifty-seven cases of infection by H1N1 virus were confirmed by RT-PCR and enrolled. Of these 57 cases, 26 had a severe H1N1 infection (group 1), and 31 had a mild H1N1 infection (group 2). Sera from 18 cases with pneumonia without H1N1 infection (group 3) were used as controls. The serum levels of 10 cytokines were measured by multiplex assay.

Results: The serum levels of IFN-α, IL-6, and IP-10 were significantly higher in H1N1 infected cases than in group 3, and levels of IL-6 and IP-10 were significantly higher in group 1 than in group 2. The level of IL-10 was significantly higher in groups 1 and 3 than in group 2. However, levels of IFN-α, TNF-α, and IL-17 were not significantly different between the three groups. IL-1, IL-4, and MIP-1α were not detectable in most patients. IP-10 and IL-6 levels were found to show negative correlations with lymphocyte count and oxygen saturation.

Conclusions: We found higher levels of cytokines (IFN-α, IL-6, IP-10) of innate immunity than those of acquired immunity in pediatric H1N1 infection. Of the cytokines found to be increased in cases with a H1N1 infection, IL-6 and IL-10 were found to be correlated with disease severity (lymphopenia and hypoxia). IP-10 and IL-6 may be important markers in pediatric H1N1 infection.

PS06
Respiratory symptoms correlate with pulmonary inflammation at a time of presumed stability in children with non-ambulant neurodisability
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Background: Children with non-ambulant neurodisability (ND) often have chronic respiratory symptoms which impact on quality of life and can result in multiple hospital admissions. Respiratory complications are a leading cause of death in this group.

Aims: Assess respiratory symptoms and broncho-alveolar lavage (BAL) inflammatory markers at a time of presumed stability.

Methods: Children with ND and healthy controls were recruited during elective surgical admission. A respiratory symptom score (LRSSQ) was completed. BAL was taken at anaesthetic induction. Neutrophil count was recorded (% total cells). IL8 and TGF-β1 were measured by ELISA. Data are expressed as median [IQR].

Results: 16 children with ND had higher LRSSQ scores than 10 controls (21 [5-46] vs. 5 [0-7], p<0.02). Children with ND had greater BAL neutrophilia (52% [31-71] vs. 4% [3-12], p=0.01) and higher IL8 (516 [2-0465] vs. 0.0232 pg/ml, p<0.05). Median TGF-β1 did not differ (0 vs.0) but the range was markedly different (0.271 vs. 0.73 pg/ml). LRSSQ score correlated with BAL neutrophilia (r=0.54, p<0.01), IL8 (r=0.49, p<0.01) and TGF-β1 (r =0.43, p=0.01). No ND patients had seen a respiratory specialist, 8/16 had undergone video-fluoroscopy (7/8 cve) and 2/16 took antibiotic prophylaxis.

Conclusions: Children with ND have a high burden of respiratory symptoms which correlate with chronic airway neutrophilia and raised inflammatory cytokines at times of presumed stability. A screening tool to identify those children who would benefit from a specialist respiratory review would be useful. Further research into the potential benefits of prophylactic therapy in this group is needed.

P507
Subclinical vitamin D deficiency and acute respiratory tract infections in children: A systematic review
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Rickets has historically been considered as a risk factor for development of pneumonia, primarily due to mechanical factors such as rib cage abnormalities and hypotonia. However, the association between vitamin D levels and acute respiratory tract infections (RTI) in children without rickets has not been thoroughly explored so far.

Aims: We aim to systematically review the available literature regarding:

- Association of low Vitamin D levels with RTI in children without clinical rickets
- Role of Vitamin D supplementation in prevention and treatment of RTI

Methods: Structured systematic literature search

Results: Seven papers on association of low Vitamin D levels and RTI were obtained. See Table 1.
related morbidity. There is some evidence from interventional studies regarding the potential role of vitamin D in prevention and treatment of RTI.

P508

Risk factors for wheezing and allergy in preschool children (PSC) after admission for acute bronchiolitis
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Introduction: Acute bronchiolitis (AB) is associated to later development of wheezing (W) and allergic sensitisation, but risk factors remain controversial. This study aimed to test the hypothesis that clinical background and features of the acute episode influences outcomes.

Methods: A cross-sectional study was performed in 71 out of 340 infants <2 year-old admitted for AB (Oct/02-Apr/04) in a tertiary teaching hospital. We conducted structured interviews, spirometry and skin-prick tests (SPT) to common inhalant allergens to 4-6 year-old and looked for demographic and clinical risk factors (male gender, born season, prematurity, exposure to smoke, nursery,older siblings, pets, family history of allergy, no breast feeding) and for the acute episode (RSV, age <6 weeks, severity) (length of hospital stay ≥6 days and time on oxygen ≥4 days) and use of steroids. Main outcomes were any episode of W and persistent wheezing (PW).

Results: W occurred in 50 (70%) of children, but only 19 (39%) referred PW. We found no differences between groups, except for steroid treatment at acute episode [W 6 (12%)/no W 8 (38%)] that was associated with a 4.5 risk reduction of wheezing (adjusted OR [IC] 0.22 [0.065-0.76]), but not for PW. PW was associated with positive SPT [PW 7 (39%); no PW 3 (10%)], adjusted OR [IC] 3.7 [1.4-10.2].

Conclusion: This study shows a high prevalence of wheezing in PSC after AB in infancy associated with atopy. The use of steroids seemed protective. We found no influence from family history of allergy, RSV infection or severity at the acute episode. These data suggests caution on using established risk factors for prognosis after an episode of BA.

P509

Effect of saltbustam on the growth, virulence and biofilm formation of pseudomonas aeruginosa
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Background: Beta-2-agonists, like saltbustam are commonly used in patients with lung disease such as cystic fibrosis where chronic infection is common. Recently saltbustam was shown to have an effect on host defence in a murine model, impairing clearance of Haemophilus influenzae from the respiratory tract [1]. To determine if saltbustam affected bacterial virulence, we investigated the effect saltbustam had on the growth and virulence of Pseudomonas aeruginosa(PA).

Aim: To determine if saltbustam affects the growth, virulence and biofilm produc tion of PA.

Methods: Clinical isolates of PA were used for experiments with and without addition of saltbustam at a range of concentrations between 0.375-100 micro gram/millilitre [2]. Viable colony counts and growth curve were performed to determine bacterial growth. Biofilm formation was studied using attachment crystal violet assay, light and advanced microscopy and electron microscopy using a cm² endotracheal tube pieces.

Results: There was no difference in the growth of PA in the presence of saltbustam. However, even at low concentrations of saltbustam (1 mcg/ml) there was a significant increase in bacterial clumping on light microscopy and increased biofilm formation on endotracheal tube sections on electron microscopy.

Conclusions: Saltbustam appears to increase biofilm formation of Pseudomonas aeruginosa. This might not imply that the use of beta-2-agonist be discouraged but suggests that a potential role in the virulence of PA must be investigated.

References:

P510

RANTES gene promoter polymorphisms -28CG and -403GA in children hospitalized with community acquired pneumonia
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The course of community acquired pneumonia in children varies between patients and it is common belief that the innate immune system of the host plays an important role to the outcome of the infection. The chemokine RANTES is an important chemotaxtractant which attracts monocytes, T cells, NK cells and eosinophils into sites of inflammation.

Aim of the study was to investigate the frequency of -28CG and -403GA RANTES polymorphisms in children hospitalized with community-acquired pneumonia.

The study involved two groups of children. The first (group A) consisted of 60 children hospitalised with pneumonia, 37 boys, aged 5.0±4.4 years and the second (group B) consisted of 135 healthy children, 60 boys, aged 9.6±6.0 years with no history of respiratory infections.

The genotypes of all subjects were determined with PCR-RFLP assay.

According to the results, only the -28CG RANTES promoter polymorphism is associated with community-acquired pneumonia in children. No association was found for the -403GA polymorphism.

P511

Diagnosis of primary ciliary dyskinesia in a Dutch cohort of 63 pediatric patients: An overview
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The diagnosis of primary ciliary dyskinesia (PCD) is difficult, as a single gold standard is lacking. The diagnosis is usually based on a combination of clinical symptoms, abnormal movement of cilia on microscopic evaluation of respiratory epithelial biopals (LM) and/or epithelial cell cultures (CLM), or identification of an ultra structural defect in the cilia by electron microscopy (EM). In order to get more insights into the diagnostic value of each of these tests, we performed a retrospective analysis in a cohort of 63 pediatric PCD patients treated within our center. Patient characteristics were as follows: mean age at diagnosis 3.8 years (range 0-18 years), males 44%, females 56% and sinus inversus 39.7%.

PCD was diagnosed based on a combination of clinical symptoms and LM in 7.9%, EM 4.8%, CLM 1.6%, LM and CLM 36.5%, LM and EM 20.6%, or LM, EM and CLM in 28.6% of the patients respectively. Abnormal beat frequency, amplitude and coordination observed in epithelial cell cultures from PCD patients, correlated with dyskinetic movement observed in the original biopsy (p <0.01). However, secondary dyskinesia is often encountered in nasal biopsies, as is illustrated by the fact that in 67 out of 136 biopsies with dyskinetic cilia obtained during the last 2 years, cell culture results were normal. In 28.6% of the PCD patients, EM findings were normal. In summary, the diagnosis of PCD cannot rely on a single technique, as both false negative and false positive results frequently occur. Epithelial cell cultures should be an integral part of the diagnostic work-up. Future studies into the genetic background may further improve diagnostic accuracy.

P512

What is the gold standard in the diagnosis of primary ciliary dyskinesia syndrome?
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Introduction: The diagnosis of primary ciliary dyskinesia (PCD) is based on the study of ciliary motility (frequency and beat pattern) using high resolution digital video and high speed, combined with ciliary ultrastructure by electron microscopy (EM.) However, this can provide false positives and negatives, so the final diagnosis is defined by the ciliary function. 

Objectives: 1. To study the real prevalence of PCD after the introduction of the technique of ciliary motility in our center (2007). 2. To set the number of cases previously classified PCD who have a normal ciliary motility.

Material and methods: Study of frequency and ciliary beat pattern: a) in patients who had been diagnosed from PCD until 2007 by screening of mucociliary clearance with 99mTc-labeled albumin and abnormal ciliary ultrastructure. b) in patients with clinical suspicion of PCD, from February 2007 to December 2010. 

Results: a) Prior to the introduction of the technique the diagnosis of PCD was made in 17 cases. With the start of movility, we obtain a normal frequency/beat pattern in 8 of them (47%), dyskinetic in 6 cases (35%), not being possible to do in 3 of them. Therefore, the diagnosis of PCD is reduced 42.8%. 37.5% of confirmed cases associated with situs inversus (S. Kartagener). b) Since 2007 we have studied 79 patients with clinical suspicion of PCD obtaining a dyskinetic pattern in 4 cases (5%), of which only 1 (25%) had anormal ciliary ultrastructure. 

Conclusions: The diagnosis of PCD based on the ciliary ultrastructure has a large percentage of false positives, so the study of ciliary motility should be considered the gold standard for diagnosis.

PS13 Is spirometry less accurate than chest computed tomography in primary ciliary dyskinesia with pulmonary deterioration? 

Marco Maglione1, Andrew Bush2, Silvia Montella 1, Carmine Mollica 3

Background: Aggressive treatment of primary ciliary dyskinesia (PCD) often stimulates ciliary motility over time. We hypothesized that spirometry may overestimate the degree of stability. 

Methods: Twenty PCD patients (median age, 11.6 yrs; range, 6.5-27.5 yrs) underwent lung function testing (RF) and HRCT at median 2.3 yrs interval. The first evaluation was made in stable state and the second because of deterioration unresponsive to standard therapy. CT scans were scored blindly by two experienced raters. 

Results: At the second evaluation, spirometry did not change while CT scores significantly worsened (p<0.01).

<table>
<thead>
<tr>
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<th>1st evaluation 2nd evaluation</th>
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<th>P</th>
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<tr>
<td></td>
<td>Spirometry (Z score)</td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td>FEF25-75</td>
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<td>FEF75-250</td>
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<td></td>
<td>HRCT scores (%)</td>
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<td>Mucus plugging</td>
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<tr>
<td></td>
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</tr>
</tbody>
</table>

Data presented as median values. 

Age was significantly correlated to CT total score (r=0.5; p=0.02). At both evaluations CT total score was related with FEV1 (Z score (r=-0.5; p=0.015 and r=-0.7; p=0.001, respectively) and FVC Z score (r=-0.6; p=0.006 and r=-0.7; p=0.001, respectively). No relationship was found between the change in CT scores and the change in spirometry.

Conclusions: In PCD patients with pulmonary deterioration, spirometry may fail to detecting worsening CT findings. Structural changes may progress with stable spirometry, calling into question the usefulness of serial spirometries to monitor PCD lung disease.

PS14 Changing characteristics of childhood non-cystic fibrosis bronchiectasis 

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In developing countries,characteristics of the chronic diseases may change in time because of changes in socioeconomic and health conditions. Our study aim was to evaluate the changing characteristics of non-CF bronchiectasis compared to our previous study (Karadağ B. et al. Respiratory 2005:72:233-8).

Patients with non-CF bronchiectasis diagnosed and followed after 2001 were recruited into the study.Long-term follow up records and lung function tests were used to evaluate the course of the disease. Collected data were compared with our previous data.

Of 100 patients included to the study,54% were male. There was an increase in the mean age of the patients compared to our previous data (12.7±4.3 vs. 7.4±3.7 years, p<0.005). Duration of symptoms prior to the diagnosis was longer (6±3.7 vs. 4±9.3±7 years, p<0.005). As similar to the previous data, in 41% of the patients no underlying etiology could be detected. There was a change in the percentages of the detected etiology (2011 vs. 2001); postinfectious (21% vs 20.7%, p=0.15), asthma (12% vs 4.5%, p=0.07), immuno deficiencies (12% vs 15.3%, p=0.55), primary ciliary dyskinesia (11% vs 6.3%, p=0.32) and foreign body aspiration (1% vs 3.6%, p=0.37). During the follow-up period, in 25% of the patients the severity of the bronchiectasis improved. The rate of surgical management decreased from 23.4% to 9% compared to previous data (p=0.005).

Clinical characteristics of childhood bronchiectasis seems to be changing in a 10-year interval. There is a tendency for a decrease in the prevalence of postinfectious bronchiectasis however uncontrolled asthma and primary ciliary dyskinesia seems to be increasing. Surgical management is used only for a small group of patients.
cost of care was £11,116 between the RSV and non respiratory groups (p=0.001) and £9,076 between the RSV and the other respiratory groups (p=0.007).

**Conclusion:** In infants born between 32-35 weeks of gestation, hospitalisation for an RSV LRTI was associated with significantly increased health related cost of care in the first two years after birth.

**P517**

**Rhinovirus-C infection in children presenting with acute respiratory infection to hospital in Brazil**

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**Introduction:** Human Rhinovirus (HRV) is a common cause of acute respiratory infection (ARI) in children. A new group of Rhinoviruses (Rhinovirus C (HRV-C)) have recently been reported. We aimed to assess the frequency of HRV groups, including HRV-C, in preschool children presenting to a paediatric emergency department with different clinical manifestations and severities of ARI.

**Methods:** Children <5 years presenting to IMIP children’s hospital, North-Eastern Brazil with ARI between March 2008 and March 2010 were recruited. They had nasopharyngeal aspirate samples collected. Clinical diagnosis and disease severity were recorded. Samples were analysed by Multiplex PCR for 18 virus and atypical bacterial pathogens. All HRV positive samples were subsequently analysed for subtype and group.

**Results:** 630 children were recruited, of whom 54% were hospitalised. Bronchitis, pneumonia, viral induced wheeze and upper respiratory tract infection were the commonest clinical diagnoses. HRV was detected in 118 samples (19%), the 4th most common infection after Respiratory Syncytial virus (32%), Adenovirus (29%) and Bocavirus (24%). Preliminary data shows 35% of HRV samples are HRV-C positive. Relationships between clinical manifestation/severity scorings will be examined once sub-typing analysis is complete.

**Conclusions:** This study underlines the importance of Rhinovirus infection in preschool children presenting to hospital with ARI. Preliminary data indicates HRV-C subtype is highly prevalent, further analysis will show whether this subtype is associated with particular clinical manifestations and severities of disease.

**P518**

**The prevalence of human rhinovirus C is low in children from the community without respiratory symptoms**

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**Background:** We have shown that human rhinovirus strains from group C (HRV-C) are associated with more frequent and severe asthma exacerbations in children. The occurrence in HRV-C in well children, with or without asthma and no current respiratory symptoms is unknown. Previous studies of patients with asthma without current symptoms have shown 12-44% had HRV detected.

**Aim:** To document the prevalence of HRV in a population of well children from the community.

**Methods:** We prospectively recruited children from the community or from the hospital with no signs of an upper respiratory infection and with no respiratory condition other than asthma. Either a nasal flocked swab or nasal wash specimen were collected from all children and tested for HRV. Viral RNA was extracted and reverse transcribed. A two-step PCR of the HRV 5’ NCR was used for detection, followed by sequencing for typing.

**Results:** We collected nasal specimens from 34 children. The age range was 8 months to 18 years (mean 11.8 years). Male to female ratio was 1:1. Twenty five (73.5%) children were recruited directly from the community. Ten (29.4%) children were recruited in spring and 13 (38.2%) in autumn, the peak seasons for HRV. Seventeen (50%) children had a nasal swab and 17 (50%) children had a nasal wash specimen. Thirteen (38.3%) had doctor-diagnosed asthma. Ten (29.4%) had atopy. We found HRV in only 6 (17.6%) of the 34 children studied. HRVA was present in 2 of the samples and HRVB in 4. All of these were detected in spring or autumn. However, no HRVC was detected.

**Conclusion:** In our community, HRV-C has a low prevalence in children who are well and without respiratory symptoms.