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Insights from the Sixth Global Experts' Meeting (GEM) on Respiratory Viruses

The year in review

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SUMMARY

Over the last year there have been more studies determining predisposition to severe bronchiolitis and its consequences. Studies have highlighted various single-nucleotide polymorphisms (SNPs) to be significantly associated with respiratory syncytial virus (RSV) hospitalisation, and a candidate gene approach demonstrated that innate immune gene SNPs had the strongest association with bronchiolitis. The impact of 'other' viruses (RSV, influenza, adenovirus, parainfluenza, rhinovirus, human metapneumovirus [hMPV], coronavirus, bocavirus, enterovirus, paraechovirus) has been investigated. In one series only children with RSV infection experienced recurrent wheezing and in another only RSV infection was associated with respiratory complications (hypoxia correlated with prolonged hospitalisation). Others have examined the long-term outcome of viral infection in infancy. The above studies and others published in the last year will be discussed.

PREDISPOSITION

Over the past year, there have been a significant number of studies determining the predisposition to severe bronchiolitis and its consequences. One study examined hospitalisations for RSV in twins born in Denmark between 1994 and 2003, totaling over 24,000 infants.[1] The concordance for RSV infection was greater for identical than fraternal twins at a rate of 0.66 versus 0.53 respectively, suggesting a genetic link. The susceptibility to severe RSV infection was influenced by genetic factors (16%), family environment (73%), and non-shared environment (11%). Other studies have highlighted various single-nucleotide polymorphisms to be significantly associated with RSV hospitalisation, but the results are not always consistent.

The RSV fusion protein activates cells through toll-like receptor 4 (TLR 4). TLR 4 polymorphisms encoding Asp299Gly and Thr399Ile have been associated with TLR 4 hyporesponsiveness and increased susceptibility to bacterial infection. In a study evaluating 105 DNA samples from high-risk patients, heterozygosity for both single nucleotide polymorphisms was associated with symptomatic RSV disease ($p<0.00001$).[2] In contrast, in another study of 236 children with RSV infection and 219 healthy controls, the Asp299Gly polymorphism was not associated with the risk of severe RSV infection and did not impact on proinflammatory cytokine production.[3]

Interleukin (IL)-18 induces interferon-gamma (IFN-γ) production and inhibits immunoglobulin E (IgE) production. Polymorphisms of IL-18 are associated with elevated serum IgE levels, atopy and asthma. In a study of 154 children with severe RSV infection and 270 controls, the -133G/C polymorphisms of IL-18 were associated with severe RSV infection ($p=0.043$). Haplotype analyses revealed associations with all six polymorphisms evaluated in the study ($p<0.001$).[4] Severe RSV infection is also associated with enhanced chemokine, including RANTES, activity which is increased in epithelial cells and nasal secretions in children with RSV. In 106 children hospitalised with RSV infection and 126 sex-matched healthy adults, the RANTES promoter gene polymorphism, -28C/C-403G/Aln1.1T/T combined genotype was commoner in cases of RSV hospitalisation than in controls ($p=0.035$).[5]

Candidate genes have been investigated in a study of 470 children hospitalised with RSV bronchiolitis, their parents, and 1,008 random population controls.[6] Single nucleotide polymorphisms with the strongest association with bronchiolitis included the vitamin D receptor (VDR) ($p=0.0017$), the JUN oncogene, which encodes the transcription factor activator protein-1 and is essential for cell cycle progression and DNA replication ($p=0.0093$), and interferon alpha 5 (IFNA5) ($p=0.0093$) and nitric oxide synthase 2 (NOS2) ($p=0.0031$) (Figure 1).[6]

Studies have also evaluated genetic susceptibility to other viral infections. In one study the occurrences of IL-10, IL-18, TLR 4, and IFN-γ polymorphisms were compared in 139 infants under six months of age hospitalised with bronchiolitis and 400 unselected blood donors.[7] Regarding the proportions of polymorphisms, infants with RSV bronchiolitis did not differ from controls, whereas those with non-RSV bronchiolitis were more likely to have an IL-10 -1082 allele G polymorphism compared with controls ($p<0.0001$).

Effects of genetic polymorphisms and late wheezing following RSV infection were studied in 101 children hospitalised for RSV lower respiratory tract infection who were followed for six years.[8] Parents kept a daily log register of respiratory symptoms during the first three years of follow-up and at six years. The IL-13 Gln allele was associated

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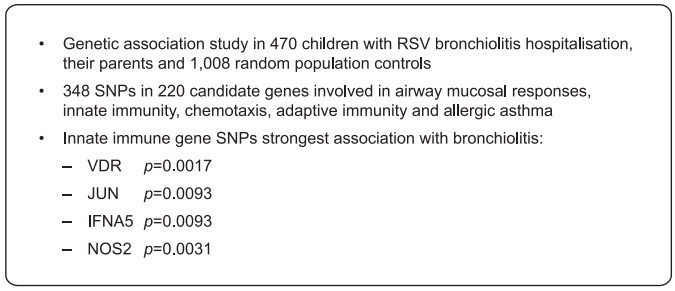

Figure 1. Candidate genes. RSV = respiratory syncytial virus; SNP = single nucleotide polymorphism; VDR = vitamin D receptor; JUN = jun oncogene; IFNA5 = interferon alpha 5; NOS2 = nitric oxide synthase 2. Source: Janssen *et al*.[6]

with late wheezing (odds ratio (OR): 3.27), but not early wheezing. This study suggests there are differences between children who develop early versus late wheezing.

DIAGNOSIS

For many years, clinicians have relied on nasal pharyngeal aspirates to diagnose RSV. With new techniques, including polymerase chain reaction (PCR), diagnosis could be made utilising saliva or throat swabs, which are more readily accessible than nasal pharyngeal aspirates, but have a lower viral load. A recent study, however, comparing the yield or positivity of nasal pharyngeal aspirates and throat swab and saliva specimens from children less than 17 years of age revealed that throat swab and saliva specimens were less sensitive than nasal pharyngeal aspirates.[9] Viruses were detected in 105 of 137 nasal pharyngeal aspirates, throat swab and saliva specimens detected 83% and 74% of viruses, respectively.

Rapid antigen detection assays have become important tools for the diagnosis of RSV and in conjunction with conventional diagnostic methods may facilitate the prompt treatment of infected patients. Results are generally available using chromatographic immunoassay (CIA) within 60 minutes and with direct fluorescent antibody (DFA) detection within about two hours. Four rapid antigen detection assays for RSV including a rapid chromatographic immunoassay (CIA), a direct fluorescent antibody (DFA) assay and two DFA assays for the human metapneumovirus (hMPV) were prospectively evaluated by comparing 515 nasopharyngeal aspirates with results from real-time reverse transcriptase (rt) PCR.[10] The CIA had poor sensitivity for RSV at 80% (90% specificity) with a turnaround time of 30 to 60 minutes. Although the RSV DFA had a 94% sensitivity and 97% specificity with a turnaround time of 30 minutes, it was highly demanding of technical time and expertise. The hMPV DFA assays had poor sensitivity of 62%, with a turnaround time of 105 minutes. Based on those results, rapid antigen testing requires further improvements before routine use in clinical practice.

OTHER VIRAL INFECTIONS

Multiplex PCR has allowed the detection of a number of viruses from one specimen. In 85 infants less than 12 months of age hospitalised for their first wheezing episode, viral infection was determined in 89.4% of nasopharyngeal aspirates using multiplex PCR detection of RSV, influenza, adenovirus, parainfluenza, rhinovirus (RV), hMPV, coronavirus, bocavirus, enterovirus, and paraechovirus.[11] RSV was the most frequently detected pathogen and only those children infected with RSV experienced recurrent wheezing, defined as a new episode of wheezing in the 6 months following the initial episode. Viral load was higher in those with recurrent wheezing.

The Asthma Multicenter Infant Cohort Study investigated the incidence and etiology of lower respiratory tract infections by home visits and viral testing in 487 infants less than one year of age.[12] The incidence rate of at least one lower respiratory tract infection was 38.7 infants/100 person-years. RSV was the most frequently isolated pathogen (44.7%). The risk of a lower respiratory tract infection was higher in infants with a maternal history of asthma (OR: 2.4) or with siblings (OR: 1.8). The risk of infection was lower in infants who were breast fed for more than 12 weeks (OR: 0.26) or who were of a lower socioeconomic class (OR: 0.16).

In a prospective two-year study of single and co-infections in 322 hospitalised infants, 46.7% of nasopharyngeal aspirates were positive for infection.[13] Molecular detection was used to identify the pathogen and genotyping of the viral strain was conducted by sequence analysis. Of the infected infants, 28% were positive for RSV (62% RSVA positive), 14.3% for hMPV, 2.2% for bocavirus, and 8.7% for coronavirus. Co-infection rates were high with RSV 23.3%, hMPV 38.3%, bocavirus 57.1%, and coronavirus 46.4%. Only RSV was associated with the respiratory complications of bacterial pneumonia and hypoxia. Hypoxia correlated with prolonged hospitalisation. In this study, mono-infection with RSV compared with RSV-hMPV co-infection resulted in longer hospitalisation and worse hypoxia ($p<0.01$).

MANAGEMENT

Unfortunately, there are very few recent studies on the management of RSV infection. A prospective study evaluated the efficacy of nasal continuous positive airway pressure (CPAP) on respiratory distress symptoms and respiratory effort in 12 infants less than three months of age with RSV-positive bronchiolitis. The patients were in the paediatric intensive care unit (PICU) and had a PCO_2 greater than 50 mmHg and clinical score of greater than 5.[14] Oesophageal pressure measurements were used to determine the pressure time product, a measure of the work of breathing. The pressure time product decreased significantly after one hour and six hours of CPAP; at baseline, the pressure time product was a mean (SE) of 952 (130) $cmH_2O.s/min$, at one hour post CPAP it was 430 (64) ($p<0.001$) and 6 hours post CPAP the pressure time product was 485 (48) ($p<0.001$). No infant required intubation and the average length of stay in the PICU was seven days. These findings suggest CPAP may be useful in some infants with bronchiolitis, but this needs testing in a randomised trial.

In a retrospective review, the efficacy of high frequency oscillatory ventilation (HFOV) was evaluated in six infants with RSV bronchiolitis who had hypercapnic respiratory failure, an overexpanded lung pattern on chest radiograph, but did not have acute respiratory distress syndrome (ARDS).[15] Use of HFOV was associated with improved oxygenation with a 50% increase in mean airway pressure from a mean 12.5 cmH_2O to 18.9 cmH_2O. There was a fall in $PaCO_2$ from 72 to 47 mmHg. All infants were extubated to CPAP or supplementary oxygen within 120 hours of treatment. There were no significant complications due to treatment with HFOV. A few infants required adrenergic support and renal function was not affected. Although not a randomised controlled clinical trial, these data suggest that RSV-induced respiratory failure with hypercapnia in some infants might respond to HFOV.

About 50% of children hospitalised for RSV are treated with antibiotics, yet secondary bacterial infection occurs in less than 1% of patients. A recent multicentre, double-blind equivalence study from the Dutch Antibiotics in RSV Trial (DART) Research Group evaluated whether antibiotics would reduce the duration of hospitalisation for patients with RSV.[16] Azithromycin (10 mg/kg/day) or placebo was administered to 100 infants less than 24 months of age with a diagnosis of RSV. After 71 infants had been randomised over a three year study period, the study was terminated due to a lack of difference in duration of hospitalisation between the azithromycin group (132 hours) and the placebo group (140 hours; $p=0.328$) and a failure of azithromycin to resolve clinical symptoms more quickly, suggesting that infants and children with RSV infection do not benefit from treatment with antibiotics.

The impact of ribavirin on the risk of asthma and allergic sensitisation was evaluated in 175 hospitalised children less than two years of age with an RSV infection.[17] Wheezing was determined by a clinician or by questionnaire. Ribavirin was administered to children with persistent respiratory distress, deteriorating cyanosis, and respiratory failure. At follow-up, cumulative asthma was significantly reduced in children receiving ribavirin (8%) compared with controls (33%;

p=0.004). Likewise, current symptoms of asthma or wheeze were significantly reduced in patients receiving ribavirin (15%) compared with controls (34%; p=0.049). The study, however, was not randomised and had only 50% follow-up, therefore the results should be interpreted with caution. The impact of ribavirin in children with RSV remains inconclusive.

OUTCOME

Premature birth, chronic lung disease in premature infants and immunodeficiency are associated with higher morbidity and mortality rates in children with RSV.[18] The German DMS RSV Ped database provided prospective multicentre documentation of RSV hospitalisations at 14 paediatric centres over six RSV seasons from 1999 to 2005. The database was interrogated and provided confirmation of the increased risk of severe RSV disease in premature infants, particularly those with chronic lung disease treated within the last six months prior to infection. Analysis of the database showed that 26% of 1,568 RSV infections were in premature infants. Mortality was 8.6% in prematurely born infants with BPD, 1.2% in prematurely born infants and 0.2% in infants born full term. Prematurity at birth, chronic lung disease with treatment within the last six months and nosocomial infection were significantly associated with a complicated course of disease including pneumonia, apnoea-bradycardia, supplementary oxygen, and PICU admission. Based on the results of the analysis, the investigators concluded that their current national guidelines for prophylaxis were appropriate for this patient population.

Another study was conducted to identify risk factors for RSV compared to all other causes of lower respiratory tract infection.[19] In a rural birth cohort of Kenyan children, children were monitored for acute respiratory infections over three RSV epidemics.[19] RSV was diagnosed by immunofluorescence (IF) assays in nasal washings at each acute respiratory infection episode. Four hundred sixty-nine children experienced 857 lower respiratory tract infections. RSV lower respiratory tract infections were associated with severe stunting, over-crowding, and the number of siblings under six years of age. A higher educational level of the primary caretaker was protective of infection.

Population-based studies have demonstrated a temporal relationship between RSV infection and invasive pneumococcal disease. A prospective population-based cohort study using registry information about hospitalisation in Denmark was conducted to further investigate the relationship between RSV infection and invasive pneumococcal disease. The registry included 17,821 RSV hospitalisations and 7,787 cases of invasive pneumococcal disease. In children less than two years of age, hospitalisation for RSV increased the risk of invasive pneumococcal disease during the 30 days after hospitalisation, with a rate ratio of 7.1. The investigators proposed the mechanism for the association was direct damage by the virus resulting in a secondary bacterial infection.

Atopy and early life viral infections have been identified as independent risk factors for the development of asthma in a community-based cohort study of 198 children at high risk for atopy (Figure 2).[20] In that study, all episodes of acute respiratory illness were captured in the first year and aspirates obtained for viral identification. Wheeze and asthma were evaluated at six months, and at two and five years. In this study, asthma was defined by a doctor diagnosis of asthma ever in the 5 years and current asthma included the presence of asthma and wheeze in the 12-months prior to the 5-year follow-up visit. Wheeze was defined as a high-pitched whistling sound coming from the chest, on expiration. Transient wheeze included wheezing episodes only in the first 3 years of life, late-onset wheeze was defined as no wheezing before 3 years of age with wheeze occurring between 3 and 5 years, persistent wheeze was defined as wheezing all 5 years of the study, and current wheeze was defined as wheeze in the 12 months prior to the 5-year follow-up visit. Analysis of aspirates revealed 69% of episodes were due to viruses, of which 10.9% were RSV and 48.3% were RV. At five years, 28.3% of children had current wheeze. Risk factors for the development of asthma where identified as wheezy infections (OR: 3.4) and/or febrile lower respiratory tract infection as infants (OR: 3.9), especially those caused by RSV or RV infections (OR: 4.1). Importantly, those risk factors were correlated with asthma only in children with sensitisation by two years of age, demonstrating an interaction between early life viral infections and atopy in the causation of asthma.

- Community based cohort study of 198 children at high risk of atopy
- All episodes of acute respiratory illness first year – postnatal aspirates for viral identification
- Wheeze and asthma at 6 months, 2 and 5 years
- Aspirates: in 69% viruses were detected
  RSV 10.9%; RV 48.3%
- At 5 years 28.3% had current wheeze:
  - Wheezy (OR 3.4) and/or febrile (OR 3.9) LRI
  - RSV/RV (OR 4.1)
- Only in children with sensitisation by 2 years of age

Figure 2. Early life respiratory viral infections, atopy and asthma. RSV = respiratory syncytial virus; LRI = lower respiratory infection; RV = rhinovirus. Source: Kusel *et al*.[20]

- 100 children aged 1–23 months hospitalised with wheeze with ARTI between 1992–1993
- 80 (24 RSV) undertook lung function tests at 10.9–13.7 years

	RSV+	RSV-	p
FVC (%)	93.7 (11)	99.5 (12.6)	0.009
FEV_1/FVC	98.4 (6.5)	92.9 (8.7)	0.033

- BHR was associated with:
  - Early sensitisation to inhaled allergens
  - Antenatal smoking
  - Early atopic dermatitis

Figure 3. Lung function and BHR 11 years after hospitalisation for bronchiolitis. RSV = respiratory syncytial virus; BHR = bronchial hyperreactivity; FVC = forced vital capacity; FEV1 = forced expiratory volume in 1 second; ARTI = acute respiratory tract infections. Source: Hyvarinen *et al*.[21]

Atopic children (n = 80) aged 1 to 23 months, who had been hospitalised within the first two years with wheeze with an acute respiratory tract infection between 1992 and 1993, were followed to a median age of 12.3 years with lung function measurements (Figure 3).[21] Twenty-four children had an RSV infection. Viral infections were determined by antigen detection in the nasopharyngeal aspirate obtained during hospitalisation and antibody determinations in paired sera. RSV was also assessed by rt PCR in frozen nasopharyngeal aspirates. Lung function measurements revealed that the children with an RSV infection compared to those without approximately 8 to 9 years prior to the measurements had evidence at follow up of a restrictive abnormality. In children who had been RSV positive, the FVC was 93.7% of predicted compared with 99.5% in children who had been RSV negative (p=0.009) and the forced expiratory volume in one second (FEV_1)/FVC was 98.4 and 92.9 in children who had been RSV positive and negative, respectively (p=0.033). In a multivariate model, the decreased FVC and essentially normal FEV_1 values suggest that RSV is an independent predictor of lower FVC and that RSV bronchiolitis is associated with a permanent abnormal and restrictive pattern of lung function.

CONCLUSION

During the past year, numerous publications have furthered our understanding of the genetic predisposition to RSV, although the data require careful consideration regarding the sample sizes, types of populations and risk factors. While there is a significant long term

airway morbidity following viral lower respiratory tract infections, the role of atopy and premorbid abnormal lung function requires further clarification. Multicentre randomised clinical trials are needed to inform management.

DISCLOSURE

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