



Studies into viral respiratory infections

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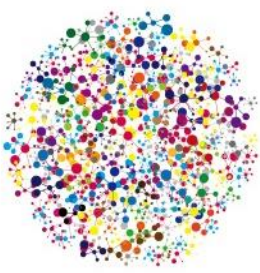
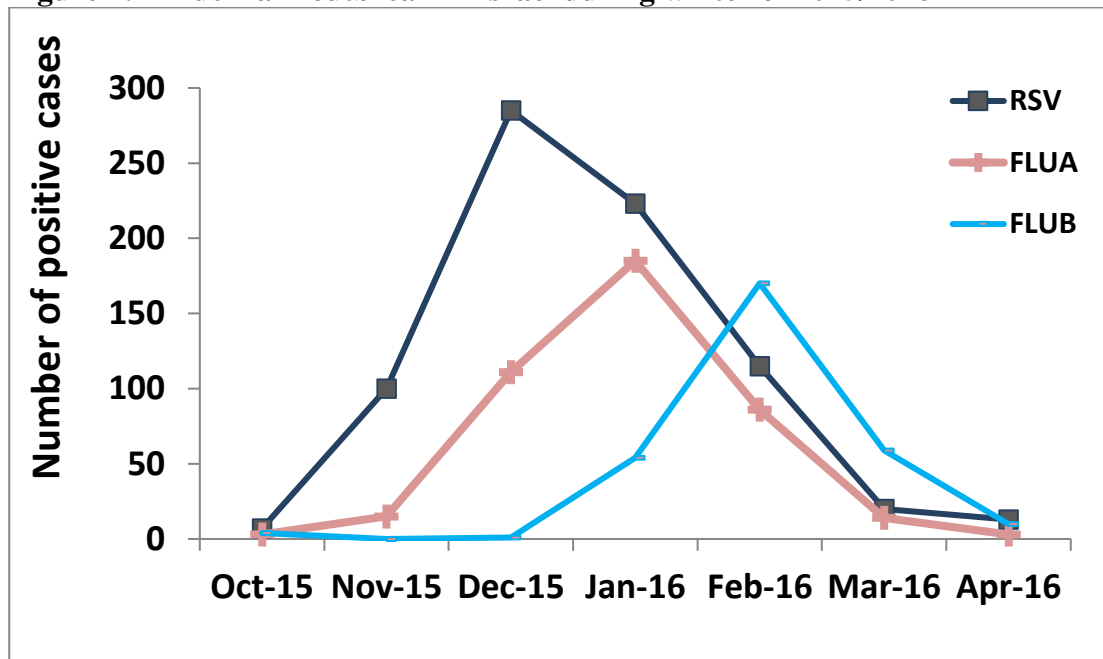
The primary objectives of the lecture are: (1) Describe the frequency and impact of viral infections and acute respiratory illnesses (ARI) among hospitalized patients and in the community; (2) Describe the analysis of respiratory viruses using in-house and commercial molecular diagnostics; (3) Estimate the effectiveness of influenza vaccination in the last two winters to prevent symptomatic influenza illness; (4) Estimate the effectiveness of pneumococcal conjugate vaccines (PCVs) on Alveolar Pneumonia in children; (5) Describe the common respiratory viruses in tonsillar tissue of children with obstructive sleep apnea; (6) Designate the nature of natural killer cell receptors, innate immune responses, in acute respiratory infections.

Despite progresses in the control of many infectious diseases, acute viral respiratory tract infections remain a leading cause of illness. Although usually self-limited in healthy adults and older children, these infections are associated with substantial loss of productive time, and cause a considerable disease burden and increased mortality among young children and the elderly worldwide. Various respiratory viruses such as respiratory syncytial virus (RSV), influenza viruses, parainfluenza viruses and adenoviruses may cause respiratory illness. In particular, RSV is the leading cause of hospitalization for respiratory tract illnesses in young children. Despite extensive diagnostic investigations, the cause of many acute upper and lower respiratory tract infections remains unknown.

The advantages of molecular testing for viral diagnosis and monitoring are now widely recognized. Qualitative molecular assays allow the early detection of viruses prior to the development of a detectable immune response or when it may be more difficult or impossible to grow the virus in culture or detect it by rapid antigen tests. An early and accurate diagnosis can affect patient care by providing timely treatment and by reducing or eliminating unnecessary hospitalization, diagnostic procedures, inappropriate use of antimicrobial agents, and associated costs.

There are some concerns about the clinical significance of viral coinfections and the persistence of viral signals beyond the acute presentation of clinical disease. However the beneficial of multiple molecular testing of pathogens overpowers the disadvantages. There is a definite need for commercial products and platforms and simpler technologies that all can use. With the introduction of multiplex-PCRs, biochips, microarrays, nanoparticles, nanobiosensors, and nanopores, simplification of highly complex molecular procedures are now a reality. We are seeing more high-performance, easy-to-use, and specimen-to-result, multiplex molecular platforms for broad-based viral detection. Thus, molecular diagnostics will become available to every laboratory and even to the point of care.

The amplitude and the types of respiratory viruses is changing year around, and from one year to the other. The burden of respiratory diseases during the winter is high in comparison to the summer. Thus, flexibility of adjustment of the respiratory panel-diagnostics is required for maximal effectivity of testing. E.g. in the winter the majority of the samples (especially in children of young age) are positive for RSV and FLUA, while in the summer finding of the two viruses are very rare, adenovirus, corona and parainfluenza virus can be found all year around.

**Figure 1: Influenza B outbreak in Israel during winter of 2015/2016**

Seasonal Flu outbreaks are linked to the circulation of influenza virus type A or B. Special attention has always been paid to influenza A epidemics; but recently, we have investigated the impact of influenza B virus epidemics, particularly the winter of 2015/2016. Community acquired influenza B epidemic peaked later than the influenza A epidemic (Figure 1), affecting both: adults and children. The antigenically different influenza B lineages that circulate during this year raised the issue of vaccine matching. Currently, in collaboration with the CDC, we investigate the efficacy of the flu-vaccines among health care personal (HCP). Interestingly, bacterial vaccines are also affected by viral infections. PCV (pneumococcal conjugate vaccines) was associated with significant declines in radiologically confirmed alveolar pneumonia (RCAP). However, seasonal variations, particularly in RSV activity, masked the impact of 7-valent PCVs, especially for young children in the first 2 years after vaccine introduction (*Emerg. Infect. Dis.*, Vol. **19**(7): 1084-91).

We applied the molecular diagnostics of respiratory viruses to test for various topics that concerns respiratory infections, e.g.: (1) do common viruses of the respiratory system prevail in the adenoids and are they involved in sleep apnea; (2) what characterizes the innate immune response, and in particular the Natural Killer cells (NK) in the upper respiratory system.

Early life viral infection is associated with neurogenic inflammation that is present in lymphoid tissues of the upper airway in children with obstructive sleep apnea (OSA). We hypothesized that viral genomic material is present in tonsils of children with OSA. Tonsils were examined for the presence of respiratory viruses' nucleic acids in children with OSA, and in children without OSA (undergoing surgery for recurrent throat infections (RI)). Twenty-four respiratory viruses were observed in 17 (50%) OSA samples. In contrast, no virus was detected in RI samples (relative frequency $P < 0.0001$). Viruses detected, based on frequency were Rhinovirus, Adenovirus, human metapneumovirus (hMPV), respiratory syncytial virus (RSV), and corona virus. Conclusions: Respiratory viruses are detected in OSA hypertrophic tonsils, suggestive of their role in the evolution of tonsillar inflammation and hypertrophy. Early life viral infections may contribute to the pathogenesis of pediatric OSA (*Pediatr Infect Dis J.* 2011; **30**(6):530-3).



The natural killer (NK) cell activating receptor NKp46/NCR1 plays a critical role in elimination of virus-infected and tumor cells. The NCR1 gene can be transcribed into five different splice variants, but the functional importance and physiological distribution of NKp46 isoforms are not yet fully understood. We examined the differential expression of NKp46 splice variants in viral respiratory tract infections and their functional difference at the cellular level. NKp46 was the most predominantly expressed natural cytotoxicity receptor in the nasal lavage of patients infected with four respiratory viruses: respiratory syncytia virus, adenovirus, human metapneumovirus, or influenza A. Domain 1-negative NKp46 splice variants (i.e., NKp46 isoform d) were the predominantly expressed isoform in nasal lavage following viral infections. NK-92 cell lines and cultured NKp46 D1-negative splice variant-expressing cells showed functional differences when interacting with targets, showing enhanced degranulation activity. To our knowledge, we provide the first evidence showing the physiological distribution and functional importance of human NKp46 splice variants under pathological conditions (*Frontiers in Immunology*. 2017 **15**;8:161).

In summary: Molecular diagnostics of respiratory viruses has become in recent years a major tool for the management of respiratory diseases, affecting the duration of hospitalization, the patient management and assisting in vaccine efficacy surveillance. These highly sensitive tools can also assist in linking between respiratory pathogen and various respiratory morbidities, of previously unidentified origin. The necessity for a simple and easy to manipulate tests for diagnosis of respiratory infections in the hospitalized patients is already a common knowledge, undoubtedly, in the future, we will witness the spread of these diagnostics tools to point of care and to the community.