# Contents

3 **PROGRAMME**

7 **ORAL ABSTRACTS**

8 Keynote lecture: Low likelihood of transmission of baloxavir-resistant influenza viruses from baloxavir-treated index patients to untreated household contacts in the BLOCKSTONE study

9 Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

13 Virus structure and replication in influenza virus, RSV and SARS-CoV-2; latest developments in influenza virus, SARS-CoV-2 and RSV molecular virology

18 Diagnostic testing in the management of acute respiratory infections in primary and secondary care

22 Co-infections in influenza, RSV disease and COVID-19

26 Innate and adaptive immunity towards influenza, RSV disease and COVID-19

30 Lessons learned from and prospects for COVID-19 vaccination

34 Pandemic threats from the animal world

38 Viral and host factors in the pathogenesis of influenza, RSV disease

44 Antiviral and immune therapy for influenza, RSV disease and COVID-19

49 Experimental medicine studies of influenza, RSV disease and COVID-19

56 Strategies for future Influenza vaccination

60 “Long Covid”: post and acute clinical sequelae of COVID-19

63 Strategies for future RSV disease vaccination

67 Why influenza is a priority for policy makers

70 Societal impact of influenza and COVID-19

73 Benefits of vaccinating healthcare workers and other risk groups

76 Novel and outstanding scientific discoveries: Late Breakers

81 **POSTER ABSTRACTS**

82 Antiviral and immune therapy for influenza, RSV disease and COVID-19

90 Benefits of vaccinating healthcare workers and other risk groups

91 Diagnostic and Intervention strategies for the management of acute respiratory infections

96 Diagnostic testing in the management of acute respiratory infections in primary and secondary care

99 Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

130 Human influenza, RSV disease and COVID-19 challenge studies

131 Innate and adaptive immunity towards influenza, RSV disease and COVID-19

138 Pandemic Preparedness Planning in Peacetime

140 Pandemic threats from the animal world

141 Science based management of epidemics and pandemics

142 Societal impact of influenza, RSV disease and COVID-19

143 Strategies for future Influenza vaccination

155 Strategies for future RSV disease vaccination

157 Viral and host factors in the pathogenesis of influenza, RSV disease and COVID-19

169 Virus structure and replication in influenza virus, RSV and SARS-CoV-2; latest developments in influenza virus, SARS-CoV-2 and RSV molecular virology

*This abstract book was published on 4 December 2021*
# Programme

## SATURDAY 4 DECEMBER

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Organiser/Chair</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:00 - 13:45 CET</td>
<td><strong>SAT</strong> Organised by <strong>Janssen</strong>: Post-COVID lockdown era: what are we facing?</td>
<td>CHAIRS: Stefan Scholten, Praxis Hohenstaufenring, Carlo Federico Perno, UniCamillus International Medical University Rome</td>
</tr>
<tr>
<td>14:00 - 15:45 CET</td>
<td><strong>SAT</strong> Organised by <strong>Sanofi Pasteur</strong>: INFLUENZA AND COVID-19 Burden of Disease and Prospects for Vaccination</td>
<td>CHAIR: Ursula Kunze, Medical University Vienna</td>
</tr>
<tr>
<td>16:00 - 17:45 CET</td>
<td><strong>SAT</strong> Funded and organised by <strong>Seqirus</strong>: Influenza during COVID-19 and beyond: Challenges, Technologies and Strategies</td>
<td>CHAIR: David Salisbury, Royal Institute of International Affairs, Chatham House</td>
</tr>
<tr>
<td>18:00 - 19:00 CET</td>
<td><strong>Opening Ceremony - Live session</strong></td>
<td>CHAIRS: Ab Osterhaus, Marco Gojejembier and Colin Russell</td>
</tr>
<tr>
<td>20:00 - 21:30 CET</td>
<td><strong>Replay of Webinar: Vaccination in a COVID-19 era</strong></td>
<td></td>
</tr>
<tr>
<td>22:00 - 23:30 CET</td>
<td><strong>Replay of Webinar: Childhood Influenza vaccination and treatment in a COVID-19 era</strong></td>
<td></td>
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<tr>
<td>Time</td>
<td>Session</td>
<td></td>
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</tr>
<tr>
<td>09:00 - 09:45</td>
<td>Recap of the day 4 December with Colin Russell and Ab Osterhaus</td>
<td></td>
</tr>
</tbody>
</table>
| 10:00 - 11:45| **KEY** COVID coagulopathy in comparison to influenza and pneumococci sepsis  
SPEAKER: Marco Goetjenbier, Erasmus MC  
**SCS** Epidemiology surveillance and modelling of influenza, RSV disease and COVID-19 including virus evolution and strain selection  
CHAIRS: Colin Russell, Academic Medical Center, University of Amsterdam, Seth Zost, Vanderbilt University Medical Center  
**SCS** Virus structure and replication in influenza virus, RSV and SARS-CoV-2; latest developments in influenza virus, SARS-CoV-2 and RSV molecular virology  
CHAIRS: Ed Hutchinson, University of Glasgow, Laura Martin-Sancho, Scripps Research  
**SPI** Why influenza is a priority for policy makers  
CHAIR: Roman Prymula, Charles University Prague |
| 12:00 - 13:45 | **KEY** On Science based management of epidemics and pandemics  
SPEAKER: Ab Osterhaus, TiHo, Hannover  
**SCS** Innate and adaptive immunity towards influenza, RSV disease and COVID-19  
CHAIR: Guus Rimmelzwaan, University of Veterinary Medicine Hannover  
**SPI** Societal impact of influenza and COVID-19  
CHAIR: Terho Heikkinen, University of Turku |
| 14:00 - 15:30 | **SAT** Organised by Roche: Impact of influenza antivirals in the post–COVID-19 era and preparing for the next pandemic  
CHAIR: Tristan Clark, University of Southampton |
| 16:00 - 17:00 | **KEY** Regulatory approvals in the US  
SPEAKER: Arnold Monto, University of Michigan  
**SAT** Poster Peek with Ab Osterhaus  
**SCS** Diagnostic testing in the management of acute respiratory infections in primary and secondary care  
CHAIRS: Michael Ison, Northwestern University, Nicole Ngai Yung Tsang, The University of Hong Kong  
**SCS** Co-infections in influenza, RSV disease and COVID-19  
CHAIR: Barbara Rath, Vienna Vaccine Safety Initiative  
**KEY** Next generation COVID-19 vaccines  
SPEAKER: Florian Krammer, Icahn School of Medicine at Mount Sinai |
| 18:00 - 19:30 | **SAT** Organised by AstraZeneca: Tackling COVID-19: Exploring the prophylaxis toolkit  
CHAIR: Flor Munoz, Baylor College of Medicine |
| 20:00 - 21:45 | **SAT** Organised by Pfizer: Evolving the Science of mRNA Vaccines: Helping to Protect Against COVID  
CHAIR: Shanti Pather, BioNTech  
**SAT** Poster Peek with Ab Osterhaus |
<p>| 22:00 - 23:30 | Replay of Webinar: COVID-19 treatment and medication |</p>
<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00 - 09:45 CET</td>
<td>Recap of the day 5 December with Ab Osterhaus and Marco Goeijenbier</td>
<td></td>
</tr>
<tr>
<td>09:00 - 09:45 CET</td>
<td><strong>KEY</strong> Influenza in the Time of COVID: Lessons for Vaccine Development</td>
<td>SPEAKER: Barney Graham, Vaccine Research Center, NIAID, NIH</td>
</tr>
<tr>
<td>10:00 - 11:45 CET</td>
<td><strong>SCS</strong> Lessons learned from and prospects for COVID-19 vaccination</td>
<td>CHAIRS: Hanna Nohynek, Finnish Institute for Health and Welfare, Margaret Lugin, BlueWillow Biologics</td>
</tr>
<tr>
<td>10:00 - 11:45 CET</td>
<td><strong>SCS</strong> Pandemic threats from the animal world</td>
<td>CHAIR: Ron Fouchier, Erasmus MC Rotterdam</td>
</tr>
<tr>
<td>10:00 - 11:45 CET</td>
<td><strong>SPI</strong> Benefits of vaccinating healthcare workers and other risk groups</td>
<td>CHAIR: Antonia Ho, University of Glasgow</td>
</tr>
<tr>
<td>12:00 - 13:00 CET</td>
<td>Poster Peek with Marco Goeijenbier</td>
<td></td>
</tr>
<tr>
<td>12:00 - 13:45 CET</td>
<td><strong>SCS</strong> Viral and host factors in the pathogenesis of influenza, RSV disease</td>
<td>CHAIRS: Peter Openshaw, Imperial College London, Katina Hulme, University of Queensland</td>
</tr>
<tr>
<td>12:00 - 13:45 CET</td>
<td><strong>SCS</strong> Experimental medicine studies of influenza RSV disease and COVID-19</td>
<td>CHAIRS: Christopher Chiu, Imperial College London, Daniel Goldhill, Imperial College</td>
</tr>
<tr>
<td>12:00 - 13:45 CET</td>
<td><strong>SPI</strong> Risk assessment and risk communication in acute respiratory virus infections</td>
<td>CHAIR: Barbara Rath, Vienna Vaccine Safety Initiative</td>
</tr>
<tr>
<td>14:00 - 15:00 CET</td>
<td><strong>SAT</strong> Organised by Roche: Advancing COVID-19 treatment: current and future perspectives on antivirals</td>
<td>CHAIR: Frederick G. Hayden, University of Virginia</td>
</tr>
<tr>
<td>16:00 - 17:30 CET</td>
<td><strong>SAT</strong> Organised by Sabin Vaccine Institute's Influenza Initiative: “The Influenza Vaccines R&amp;D Roadmap: Framework for a Flu-Free Future”</td>
<td>INTRODUCTION: Stacey Knobler, Vice President of Vaccine Innovation, Sabin Vaccine Institute, Mike Osterholm, Director, Center for Infectious Disease Research and Policy (CIDRAP)</td>
</tr>
<tr>
<td>18:00 - 18:30 CET</td>
<td><strong>KEY</strong> Viral and host factors in the transmission of influenza and Covid-19</td>
<td>SPEAKER: Wendy Barclay, Imperial College London</td>
</tr>
<tr>
<td>18:00 - 19:45 CET</td>
<td><strong>SCS</strong> Antiviral and immune therapy for influenza, RSV disease and COVID-19</td>
<td>CHAIR: Frederick G. Hayden, University of Virginia</td>
</tr>
<tr>
<td>18:00 - 19:45 CET</td>
<td><strong>SCS</strong> Strategies for future Influenza vaccination</td>
<td>CHAIRS: Florian Krammer, Icahn School of Medicine at Mount Sinai, Nicholas Wu, University of Illinois at Urbana-Champaign</td>
</tr>
<tr>
<td>18:00 - 19:45 CET</td>
<td><strong>SPI</strong> Global health perspectives on acute respiratory virus disease and how to ensure equitable access</td>
<td>CHAIR: Ann Moen, Centers for Disease Control</td>
</tr>
<tr>
<td>18:30 - 19:00 CET</td>
<td><strong>KEY</strong> Antibodies against SARS-CoV-2: A Global Collaboration</td>
<td>SPEAKER: Erica Ollmann Saphire, La Jolla Institute</td>
</tr>
<tr>
<td>20:00 - 21:30 CET</td>
<td>Replay of Webinar: RSV disease in a COVID-19 era</td>
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<tr>
<td>22:00 - 23:30 CET</td>
<td>Replay of Webinar: Flu and COVID-19 booster vaccinations: where do we go?</td>
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<tr>
<td>09:00 - 09:45</td>
<td>Recap of the day 6 December with Marco Goeijenbier and Colin Russell</td>
<td></td>
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<tr>
<td>10:00 - 11:00</td>
<td>Plenary session dedicated to the work of Young Scientists</td>
<td></td>
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<tr>
<td></td>
<td>CHAIR: Marco Goeijenbier</td>
<td></td>
</tr>
<tr>
<td>11:00 - 11:30</td>
<td><strong>KEY</strong> Likelihood of transmission of baloxavir-resistant influenza viruses from baloxavir-treated index patients to untreated household contacts in the BLOCKSTONE study</td>
<td></td>
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<tr>
<td></td>
<td>SPEAKER: Aeron Hurt, Roche</td>
<td></td>
</tr>
<tr>
<td>11:00 - 12:00</td>
<td>Poster Peek with Colin Russell</td>
<td></td>
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<tr>
<td>11:30 - 12:00</td>
<td><strong>KEY</strong> Human challenge studies: limitations and strengths</td>
<td></td>
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<tr>
<td></td>
<td>SPEAKER: Christopher Chiu, Imperial College London</td>
<td></td>
</tr>
<tr>
<td>12:00 - 12:30</td>
<td><strong>KEY</strong> Vaccine development and ensuring equitable access to those most in need</td>
<td></td>
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<tr>
<td></td>
<td>SPEAKER: Melanie Saville, CEPI</td>
<td></td>
</tr>
<tr>
<td>12:00 - 13:45</td>
<td><strong>SCS</strong> &quot;Long Covid&quot;: post and acute clinical sequelae of COVID-19</td>
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<tr>
<td></td>
<td>CHAIR: Susanne Herold, UKSIM Gießen</td>
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<tr>
<td>12:00 - 13:45</td>
<td><strong>SCS</strong> Strategies for future RSV disease vaccination</td>
<td></td>
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<tr>
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<td>CHAIR: Rebecca Cox, University of Bergen</td>
<td></td>
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<tr>
<td>12:00 - 13:45</td>
<td><strong>SPI</strong> Pandemic Preparedness Planning in Peacetime</td>
<td></td>
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<tr>
<td></td>
<td>CHAIR: Ab Osterhaus, TIHo, Hannover</td>
<td></td>
</tr>
<tr>
<td>14:00 - 15:45</td>
<td>Organised by Janssen: Shining light on the spectrum of RSV disease burden in adults</td>
<td></td>
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<tr>
<td>16:00 - 17:45</td>
<td>Novel and outstanding scientific discoveries: Late Breakers</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CHAIR: Sylvie van der Werf, Institut Pasteur</td>
<td></td>
</tr>
<tr>
<td>18:00 - 18:30</td>
<td>Recap of the day with Ab Osterhaus and Colin Russell</td>
<td></td>
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<tr>
<td>18:30 - 19:00</td>
<td>Closing Ceremony</td>
<td></td>
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<tr>
<td></td>
<td>CHAIRS: Ab Osterhaus, Colin Russell, Marco Goeijenbier</td>
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</tbody>
</table>
Oral abstracts
Low likelihood of transmission of baloxavir-resistant influenza viruses from baloxavir-treated index patients to untreated household contacts in the BLOCKSTONE study

**Topic:** Viral and host factors in the transmission of influenza, RSV disease and COVID-19

Joanne Harding (1), Corrado Bernasconi (2), Sarah Williams (1), Steffen Wilcum (2), Masahiro Kinoshita (3), Takeki Uehara (3), Aeron Hurt (2)

1: Roche Products Ltd, Welwyn Garden City, UK;
2: F. Hoffmann-La Roche Ltd, Basel, Switzerland;
3: Shionogi & Co., Ltd., Osaka, Japan

**BACKGROUND**

Treatment-emergent resistance to influenza antivirals is a consequence of antiviral treatment and may become of concern if such antiviral-resistant viral variants transmit and circulate within the community. Baloxavir is an endonuclease inhibitor approved for influenza treatment and prophylaxis, and substitutions in the viral polymerase acidic (PA) gene at position 38 (PA/I38X) can lead to baloxavir resistance. Here, with a post-hoc analysis, we examined the likelihood of PA/I38X virus transmission relative to wild type (WT) virus by comparing the number of PA/I38X transmission events in the phase 3 BLOCKSTONE trial (EudraCT: 2020-000696-20; Ikematsu et al. NEJM 2020) with those predicted according to data across baloxavir clinical trials.

**METHODS**

BLOCKSTONE evaluated baloxavir vs placebo as post-exposure prophylaxis in household contacts (HHCs) of index patients (IPs) in Japan. IPs received standard-of-care antivirals. The current sub-analysis included baloxavir-treated IPs with ≥1 HHC who received placebo, were influenza PCR-negative at baseline and, if infected, had a matching infection with the same virus type/subtype as the IP. The actual number of IPs shedding PA/I38X viruses was unknown as swabs were not collected after Day 1; therefore, a prediction was derived from pooled clinical trial data according to age group (< / ≥12 years) and virus type/subtype (A/H1N1, A/H3N2, B) based on the baloxavir phase 2 and 3 global and Japanese studies. Using both the predicted resistance rate in IPs and the observed transmission rate for any influenza virus in BLOCKSTONE, the expected incidence of PA/I38X among HHCs (sampled systematically) was derived (assuming PA/I38X and WT viruses have equivalent fitness/transmissibility). This was then compared with the actual observed frequencies of PA/I38X in HHCs in BLOCKSTONE to address whether PA/I38X viruses are more or less likely to transmit than WT viruses.

**RESULTS**

Overall, 152 baloxavir-treated IPs and 172 HHCs were included. PA/I38X frequency from pooled clinical trial data led to the prediction that 19.7 IPs from BLOCKSTONE shed PA/I38X viruses. Of the n=152 baloxavir-treated IPs, 24.3% (n=37) caused an infection within their households, meaning the predicted number of transmission events of PA/I38X viruses (assuming for the purposes of this analysis, equal transmission of WT and PA/I38X) was 4.80 (19.7 [estimated number of IPs with resistance] x 24.3% [transmission frequency in households]). However, zero cases of PA/I38X were observed among evaluable HHCs who received placebo.

**CONCLUSIONS**

Under the assumption that WT and PA/I38X viruses transmit with equal fitness, the likelihood of observing zero PA/I38X transmission events when 4.80 cases is predicted is only 0.77%. These data suggest that PA/I38X viruses may transmit less frequently than WT viruses, but further validation is needed.
Human and avian influenza A viruses in Cambodia during the SARS-CoV-2 pandemic, 2020-2021

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Jurre Y. Siegers, Erik A. Karlsson
Institute Pasteur du Cambodge, Cambodia

**BACKGROUND**
Introduction of non-pharmaceutical interventions to control COVID-19 in early 2020 coincided with a continuing global decrease in human influenza circulation. The majority of influenza detections in 2020-2021 have been in Southeast Asia and Western Africa. Unlike human seasonal viruses, avian influenza viruses (AIV) continue to circulate unabated in poultry in live bird markets (LBM). Historically, Cambodian LBMs have high levels of AIV circulation. In addition to A(H5) and A(H7), A(H9N2) is detected in a large percentage of Cambodian chickens, and, due to their broad host range, global distribution, and reassortment capability, pose a moderate pandemic risk. Therefore, maintenance of influenza surveillance systems, both in humans and at the human-animal interface, are crucial for monitoring emerging and potential pandemic influenza viruses and to ensure the availability of vaccine donor strains.

**METHODS**
We describe virological findings from human seasonal and LBM surveillance in Cambodia and neighboring countries. Genetic and antigenic characteristics of Cambodian human influenza viruses were assessed to determine the degree of vaccine-match with the recommended A(H3N2) influenza vaccine. In addition, a “One Health” outbreak investigation including epidemiology, serology, virology, and phylogeny was performed following a novel AIV detection in human sentinel influenza-like illness (ILI) surveillance.

**RESULTS**
Between July and November 2020, a regional influenza A(H3N2) epidemic occurred in Cambodia that comprised genetically and antigenically similar viruses, but distinct from the WHO recommended influenza A(H3N2) vaccine virus component for 2020-2021 Northern Hemisphere season. Phylogenetics revealed multiple possible virus migration events between Cambodia and bordering countries (Laos PDR and Vietnam) immediately following the Cambodian outbreak.

Unlike the low incidence of human seasonal influenza worldwide, A(H5Nx), A(H7Nx), and A(H9Nx) AIVs continue to circulate in Cambodian poultry populations at 25%, 10%, and 50%, respectively. In February 2021, Cambodia detected the first human avian influenza A(H9N2) virus infection through and launched a “One Health” investigation. One chicken sample from the infected child’s house was positive for A(H9N2) virus and genetically similar to the human virus. Phylogenetic analysis revealed that the hemagglutinin genes of both viruses clustered with G132/BJ1 lineage viruses from Southern China from 2018. Neuraminidase genes clustered to G9/BJ44 lineage viruses from Laos in 2019. Serology identified no recent A(H9N2) infections in 43 close contacts.

**CONCLUSIONS**
In February 2021, a virus from the Cambodian A(H3N2) outbreak was recommended by WHO as the prototype virus for inclusion in the 2021-2022 Northern Hemisphere influenza vaccine. Influenza will return in a post-COVID era. Early detection and control of influenza in both humans and animal populations is critical to reduce future risk, enhance biosafety, ensure candidate vaccines, and monitor antiviral treatment efficacy necessary to mitigate the risks of continued evolution of human seasonal strains or AIVs with increased mammalian adaptation or human-to-human transmission potential.
**Influenza-associated hospitalisation rates: national burden estimates for 40 countries worldwide, the Burden of Influenza and RSV Disease (BIRD) study**

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

John Paget (1), Lisa Staadegaard (1), Xin Wang (2), You Li (2), Jejanneke van Summeren (1), Tayma van Pomeren (1), Michel Dückers (1), Sandra Chaves (3), Emily Johnson (4), Cedric Mahe (3), Harish Nair (2), Cécile Viboud (5), Peter Spreeuwenberg (1)

1: Nivel, The Netherlands; 2: Centre for Global Health, Usher Institute, University of Edinburgh, Edinburgh, UK; 3: Foundation for Influenza Epidemiology, Fondation de France, Paris, France & Vaccine Epidemiology and Modelling Department, Sanofi Pasteur; 4: Institute of Health Metrics and Evaluation, University of Washington, Seattle, USA; 5: Fogarty International Center, National Institutes of Health, Bethesda, USA

**BACKGROUND**

Efforts to estimate the disease burden of influenza have gathered pace in recent years, especially since the publication of the WHO handbook (2015): ‘A Manual for Estimating Disease Burden Associated with Seasonal Influenza’. The WHO estimates that annual (seasonal) influenza epidemics result in 3 to 5 million cases of severe illness (often associated with hospitalisations) per annum. The goal of our study was to summarize and better understand influenza-associated hospitalisation estimates around the world at a national and global level.

**METHODS**

We performed a systematic literature review of English- and Chinese-language papers published since 1995 providing influenza-associated hospitalisation estimates. The extracted data were analysed using a (meta-analysis) logit-logistic regression model to: a) develop national and global estimates by age group (all ages, 0-4 and 65+ years) and b) identify the role of five factors (rate-/modelling-based estimates, the outcome variable studied, laboratory confirmed or not, regional/national estimates and single/multiple season estimates) on hospitalisation estimates.

**RESULTS**

The literature review identified 134 papers (7 in Chinese) from 46 countries. A total of 710 influenza-associated hospitalisation rates from 40 countries were included in our analysis. The national influenza-associated hospitalisation rates (all ages) varied widely, ranging from 122.1 (95% CI: 41.5-358.5) per 100,000 in India to 11.7 (CI: 3.8-36.3) in New Zealand. The overall average hospitalisation rate was 40.1 (CI: 23.3-69.1) per 100,000 per season, with rates varying substantially by age: 137.8 (CI: 70.6-268.7) in children (aged 0-4) and 69.6 (CI: 40.7-118.8) in the elderly (aged 65+). For the all age-hospitalisation analysis, hospitalisation rates varied significantly (p<0.05) by type of study (rate-based higher), whether the hospitalisations were laboratory confirmed (if yes, lower), and the number of seasons (single seasons higher). With the exception of the US, where hospitalisation rates were highest in the elderly, all other countries had higher influenza-associated hospitalisation rates in children (aged 0-4) compared to those 65+ years. For example, in mainland China the average hospitalisation rate per 100,000 in children was 364.0 (CI: 110.5-1191.1) compared to 157.1 (CI: 45.9-535.6) in the elderly; and in the UK it was, respectively, 191.7 (CI: 84.0-436.8) and 107.1 (CI: 41.8-274.2).

**CONCLUSIONS**

Our study helps to better define the disease burden pyramid of influenza for hospitalisations at a national and global level, which is very important for public health planning and messaging. Further, more extensive analyses are required to assess the impact of other factors on the hospitalisation estimates (e.g. impact of influenza vaccination, predominant strain), and efforts to harmonise the estimation methods should be encouraged. Our study highlights the high rates of influenza-associated hospitalisation in children aged 0-4 years and our global estimate of 40.1 per 100,000 population is equivalent to roughly 3 million hospitalisations per annum, which is consistent with the WHO estimate.
Mapping the antigenic drift of N1 Neuraminidase in A(H1N1) strains from 1977-1991 to understand the childhood imprinting of anti-Neuraminidase antibodies

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Pavithra Daulagala (1), Louise Yung (1), Susan Chiu (2), Kathy Leung (1), Rodney Daniels (3), Joseph Wu (1), Hui-Ling Yen (1), Malik Peiris (1)

1: WHO Collaborating Centre for Infectious Disease Epidemiology and Control, School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR;
2: Department of Paediatrics and Adolescent Medicine, Queen Mary Hospital and Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong Special Administrative Region, China;
3: The Francis Crick Institute, The Crick Worldwide Influenza Centre, WHO Collaborating Centre for Reference and Research on Influenza, Midland Road, London

**BACKGROUND**

Influenza virus seasonal epidemics are caused by antigenic drifts of viral surface glycoproteins hemagglutinin (HA) as well as neuraminidase (NA) which could emerge discordant in time from each another. Although antigenic drifts of HA have been well characterised, there is limited information on drifts of NA. Here we characterised the drifts of NA from 1977-1991. We then used a panel of antigenically distinct NA from 1977-2015 in a sero-epidemiological study to investigate the relationship between age and neuraminidase inhibition (NI) titers.

**METHODS**

A two-way analysis was carried out to identify the antigenic drifts of NA from 1977-1991 using Enzyme Linked Lectin Assay (ELLA). H6N1 viruses generated using NA from the H1N1 vaccine strains were tested against homologous as well as heterologous ferret anti-sera raised against wild-type H1N1 viruses, in parallel. A 4-fold or higher titer difference between two strains in both ways was considered as an “antigenic drift” . The antigenically distinct NAs identified from the two-way analysis together with the previously known antigenic drifts identified from 1991-2015 were then used to test the NI responses in an age-stratified panel of sera (n=130) aged 5-70 years in Hong Kong. The sera were collected from 2020-2021 during a period with reduced influenza activity.

**RESULTS**

Among the 5 WHO recommended vaccine strains from 1977 to 1991, only A/USSR/90/1977, A/Singapore/56/1986 and A/Texas/36/1991 viruses were antigenically distinct in the NA protein with >4-fold differences in the two-way NI analysis. In the age-stratified cross-sectional study, when the NI titer were analysed by age at the time of testing, the strains that circulated during the childhood of the individuals gave the highest NI titers. Those over 45 years of age at the time of testing still maintained NI titers of ≥640 for USSR/77 even though it has been replaced by later drift variants many decades ago.

**CONCLUSIONS**

The results support the discordant antigenic change in the HA and NA proteins of seasonal influenza viruses. The cross-sectional sero-epidemiology study showed evidence of imprinting for N1 NA. The higher NI titers observed for strains circulating during childhood could be as a result of antigenic seniority with repeated back-boosting of older N1 variants with subsequent infections or vaccinations. In spite of reduced influenza activity due to COVID-19 in 2020 and 2021, we still observed high N1 NI titers to the older strains showing evidence that NA antibodies could be long-lasting.
Influenza A virus undergoes compartmentalized replication in vivo dominated by stochastic bottlenecks

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Katherine Amato (1), Luis Haddock III (2), Katarina Braun (2), Victoria Meliopoulos (3), Brandi Livingston (3), Rebekah Honce (3), Grace Schaack (1), Emma Boehm (2), Christina Higgins (1), Gabrielle Barry (2), Katia Koelle (4), Stacey Schultz-Cherry (3), Thomas Friedrich (5), Andrew Mehle (1)

1: Microbiology & Immunology, University of Wisconsin-Madison, United States of America; 2: Pathobiological Sciences, University of Wisconsin School of Veterinary Medicine; 3: Department of Infectious Diseases, St. Jude Children’s Research Hospital; 4: Department of Biology, Emory University; 5: Pathobiological Sciences, University of Wisconsin School of Veterinary Medicine, Wisconsin National Primate Center

**BACKGROUND**

Transmission of influenza A viruses (IAV) between hosts is subject to numerous physical and biological barriers that impose genetic bottlenecks, constraining viral diversity and adaptation. Genetic bottlenecks within individual hosts may also shape evolutionary pathways taken during infection and subsequent transmission. High-resolution characterization of the nature and stringency of intrahost bottlenecks during IAV infection has not been previously possible.

**METHODS**

Naturally occurring genetic diversity in IAV is too low to characterize within-host bottleneck dynamics at high resolution. To overcome this limitation, we created highly diverse IAV libraries bearing molecular barcodes on two independent gene segments, enabling quantitative tracking of unique virus lineages within hosts. Validated libraries were incredibly rich and diverse with a high degree of evenness throughout the population. These libraries were paired with new analytical techniques to track adaptation during tissue culture passage, infections in mice, and dissemination throughout the respiratory tract in ferrets.

**RESULTS**

Our results show that IAV infection in lungs is characterized by multiple within-host bottlenecks that result in “islands” of infection in lung lobes, each with genetically distinct populations. We performed site-specific inoculation of barcoded IAV in the upper respiratory tract of ferrets and tracked viral diversity as infection spread to the trachea and lungs. We demonstrated compartmentalized replication of discrete barcoded populations within the lobes of the lung. Replication in vivo revealed little evidence of positive selection. This contrasts with positive selection detected during tissue culture adaptation. Barcoded libraries revealed that selective sweeps in culture are seeded by a de novo variant in HA that arises in multiple lineages, but only a single adaptive lineage rose to high frequency to dominate the population.

**CONCLUSIONS**

Bottlenecks stochastically sampled individual viruses from the upper respiratory tract or the trachea that became the dominant genotype in a particular lobe. These populations are shaped strongly by founder effects, with no evidence for positive selection. The segregated sites of replication highlight the jackpot-style events that contribute to within-host influenza virus evolution and may account for low rates of intrahost adaptation.
Genomic assembly of live attenuated influenza vaccine viruses is mediated via intersegmental RNA:RNA interactions

Topic: Virus structure and replication in influenza virus, RSV and SARS-CoV-2; latest developments in influenza virus, SARS-CoV-2 and RSV molecular virology

Sameer Ayaz (1), David Chapman (1), David Bauer (2), Oliver Dibben (1)

1: Flu BPD, Biopharmaceuticals Development, R&D, AstraZeneca, Liverpool, United Kingdom; 2: RNA Virus Replication Laboratory, The Francis Crick Institute, 1 Midland Rd, London, United Kingdom

BACKGROUND
Recombinant strains for AstraZeneca’s Live Attenuated Influenza Vaccine (LAIV, FluMist® or Fluenz®) are produced with antigenic characteristics derived from the HA and NA genes from circulating wild-type influenza viruses. Increased non-infectious particle shedding of LAIV strains with reduced replicative fitness and vaccine effectiveness (VE) has been observed in vivo. One explanation could be that inefficient genomic assembly increases the number of virus particles lacking the complete eight segment genome. Evidence is emerging that intersegmental RNA:RNA base pair interactions could play a role in the genome assembly process. Therefore, we hypothesised that the assembly of the LAIV genome is mediated by intersegmental RNA:RNA base pair interactions.

METHODS
Sequencing of Psoralen Linked And Selection of Hybrids (SPLASH) was utilised to describe global RNA:RNA base pair interactions for LAIV strains of different subtypes (A/Ann Arbor/06/1960, A/New Caledonia/20/1999, and A/Bolivia/SSS/2013). The most frequent RNA interactions were identified from this heterogenous set of strains and characterised by modifying the model A/New Caledonia/20/1999 LAIV strain (with high VE) with synonymous mutations at these loci and investigating the effect on genomic assembly and infectious virus formation.

RESULTS
Comparison of HA segment interactions between LAIV strains showed no common interactions, and the partner segment to the HA varied across strains. Three of the most frequent interactions from this heterogenous set of strains, located at the fusion peptide (FP) and transmembrane domains (TM1 and TM2) of the HA protein, were identified and investigated.

Triple synonymous mutations modifying FP, TM1, and TM2 of the A/New Caledonia/20/1999 HA showed an approximately 1,000,000-fold reduction in infectious virus when compared to the parental strain. Generation of single TM1 and TM2 mutants reduced infectious virus titre to equivalent levels as the triple mutant. Interestingly, the FP mutant showed >0.5 Log10 TCID50/ml increase in infectious virus titre.

Gene segment abundance was measured to investigate if these infectious virus titre changes corresponded to a genome packaging change. Triple FP/TM1/TM2 and single TM1 and TM2 mutants all showed significant reductions (>2 fold) in PA, NP, and NA segments relative to the parent strain. However, no change in the mutated HA segment packaging was observed.

CONCLUSIONS
Our data show that intersegmental RNA interactions of LAIV are dependent on the sequence of the HA segment. These interactions show both positive (FP) and negative (TM1 and TM2) effects on genome assembly. Furthermore, TM1 and TM2 interaction loci fall within previously described packaging sequences indicating that RNA:RNA base pair interactions facilitate assembly. Reduced packaging of non-mutated segments was observed suggesting that RNA could be remodelled, having a ‘knock on’ effect on packaging of other segments. This work highlights the importance of RNA interactions on influenza genome assembly and could provide a novel approach to improving the phenotype of LAIV.
Adaptive evolution of PB1 from A(H1N1)pdm09 towards an enhanced compatibility between PB1 and HA

Topic: Virus structure and replication in influenza virus, RSV and SARS-CoV-2; latest developments in influenza virus, SARS-CoV-2 and RSV molecular virology

Filipe Almeida (1), Luís A. Santos (1), João Trigueiro-Louro (1,2), Helena Rebelo-de-Andrade (1,2)

1: Antiviral Resistance Lab, Research & Development Unit, Infectious Diseases Department, Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisbon, Portugal;
2: Host Pathogen Interactions Unit, Research Institute for Medicines, iMed-ULisboa, Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal

BACKGROUND
The role of the polymerase basic protein 1 (PB1) in viral fitness has been mostly attributed to its function in viral replication. However, there is also evidence that the functional compatibility between PB1 and antigenic proteins plays an important role in the overall viral fitness. It was described that PB1 retain traces of interspecies transmission and adaptation. Phylogenetic analyses described genomic mutations L298I, R386K and I517V in PB1 to have putatively enhanced the compatibility between PB1 and hemagglutinin (HA) in A(H1N1)pdm09, and were proposed to have an impact in viral fitness. This study aims to evaluate the phenotypic expression of the reversal of these mutations in the PB1 of A(H1N1)pdm09 and infer the putative role of these residues in the virus overall fitness and adaptation.

METHODS
The complete gene library of A/Portugal/82/2009 (H1N1) assembled in pCIPolISapIT was used as A(H1N1)pdm09 prototype strain. Site-directed mutagenesis was performed to generate mutated PB1 genes with L298I, K386R and V517I mutations. The recombinant wild type (wt) virus bearing wt PB1 and the recombinant viruses bearing each individual mutated PB1, or the PB1 triple mutant, were generated by plasmid-based reverse-genetics. The phenotypic impact of the mutations in viral growth was evaluated by plaque forming unit and neuraminidase assays, at different timepoints post-infection.

RESULTS
Preliminary results suggest that reverting the adaptive mutations in PB1 resulted in a decrease in viral growth of the A(H1N1)pdm09 recombinant PB1 mutant viruses, in comparison with the recombinant wt. The decrease in viral growth was also accompanied by a reduction in neuraminidase activity. The mutations appear to have a cumulative effect as the recombinant virus bearing PB1 triple mutant exhibited lower infectious titers and neuraminidase activity than each individual recombinant PB1 mutant and the recombinant wt.

CONCLUSIONS
Our preliminary data indicate that the adaptive evolution occurred in the PB1 of A(H1N1)pdm09 virus may have enhanced the functional compatibility between the PB1 and HA, leading to an improved overall viral fitness. Further experiments will be directed to determine the possible effect of the mutations in the hemagglutination titer and in the activity of the ribonucleoprotein complex.

Keywords influenza virus, viral fitness, PB1, HA

Acknowledgments This research has been funded by Fundação para a Ciência e Tecnologia grant PTDC/SAU-INF/30729/2017.
Lab adapted vs contemporary RSVs: single amino acid changes modulate F protein mediated cell fusion

**Topic:** Virus structure and replication in influenza virus, RSV and SARS-CoV-2; latest developments in influenza virus, SARS-CoV-2 and RSV molecular virology

Alina Schadenhofer, Wendy K. Jo, Albert D.M.E. Osterhaus, Martin Ludlow

University of Veterinary Medicine Hannover, Germany

**BACKGROUND**

Respiratory syncytial virus (RSV) is the world leading cause for infant pneumonia and one of the main causes of lower respiratory tract infection in older adults and immunocompromised individuals, causing high morbidity and significant mortality in these populations. The fusion (F) protein represents an attractive target for therapeutics and vaccine development due to its role in virus entry and cell-to-cell spread. However, basic, and translational research studies in this area have largely used prototypic RSV-A strains, such as A2 or Long, which have a complicated passage history and exhibit different phenotypes in in vitro and in vivo models in comparison to contemporary clinical isolates. We have therefore quantified the cell-to-cell fusion induced in Vero cells by unmodified or modified codon-optimized F proteins derived from RSV-A and two recent clinical isolates of RSV-A (0594) and -B (9671).

**METHODS**

Expression plasmids based on the pCG vector containing the codon optimized F protein open reading frames of RSV-A, RSV-B have been described previously. Nucleotide changes encoding for desired amino acid substitutions were introduced into the codon optimized plasmids by Q site directed mutagenesis (NEB). Cell fusion assays were performed in Vero cells by transfection of 1.5ug plasmid encoding for each fusion construct. Quantitative cell fusion assays were performed using a β-galactosidase (β-gal) complementation assay via transfection and mixing of two populations of Vero cells with the omega-subunit of β-galactosidase alone or the alpha-subunit of β-galactosidase and the RSV-F-expression plasmid of interest respectively. Enzymatic activity was analyzed using a Tecan Infinite F pro reader.

**RESULTS**

Introduction of the A2 specific amino acid change E66K into the RSV-A-0594 F protein resulted in enhanced cell-to-cell fusion, but a similar effect was not observed with the RSV-B-9671 F protein. Mutagenesis of the RSV-A2 F protein to contain K66E resulted in only a slight decrease in cell fusion. Additional RSV-A2 specific amino acid changes located in the F2 domain (S25G, P101Q) also resulted in increased cell fusion in the context of the RSV-A-0594 F protein. Conversely, the Palivizumab escape mutation S275L resulted in a large decrease in cell-to-cell fusion upon introduction into the RSV-A-0594 and RSV-B-9671 F proteins. This phenotypic change could only partially be rectified by introducing the E66K mutation into the RSV-A-0594 F protein, but not the RSV-B-9671 F protein.

**CONCLUSIONS**

In summary, we show that the ability of the RSV F protein to induce cell-to-cell fusion in an immortalized cell line is highly strain dependent and single amino acid changes can have a significant impact on modulating fusion activity.

**Reference**

Coinfection with influenza A virus and respiratory syncytial virus generates a novel class of viral particles

**Topic:** Virus structure and replication in influenza virus, RSV and SARS-CoV-2; latest developments in influenza virus, SARS-CoV-2 and RSV molecular virology

**Joanne Haney (1), Swetha Vijayakrishnan (1), Kieran Dee (1), James Streetley (2), Mairi Clarke (2), Margaret Mullin (3), Verena Schultz (1), Daniel M Goldfarb (1), Stephen D Carter (1), David Bhella (1), Pablo R Murcia (1)**

1: MRC Centre for Virus Research, University of Glasgow, United Kingdom; 2: Scottish Centre for Macromolecular Imaging, University of Glasgow, UK; 3: Glasgow Imaging Facility, Institute of Infection, Immunity and Inflammation, University of Glasgow, UK

**INTRODUCTION**

Respiratory viruses are the cause of significant disease burden and coinfections with more than one virus constitute ~10-30% of viral respiratory infections. Many co-circulating respiratory viruses share tropism within the respiratory tract, which results in potential for infection by two unrelated viruses within the same cell in a coinfected host. This provides opportunity for direct contact between coinfecting viruses, however, sources virus-virus interactions in the respiratory epithelium are poorly characterised.

**METHODS**

We developed a model using human lung cells (A549) to study coinfection between influenza A virus (IAV) and respiratory syncytial virus (RSV). We analyzed coinfected cells using a pipeline combining high-resolution imaging methodologies, including super-resolution confocal microscopy, live cell imaging and cryo-electron tomography. We also carried out coinfections in primary differentiated human bronchial epithelial cell (hBEC) cultures to determine the likelihood of viral interactions in the differentiated airway epithelium.

**RESULTS**

Using super-resolution confocal microscopy, we examined virus particles budding from coinfected cells. We identified filamentous structures that incorporate glycoproteins from both viruses in distinct patches along the filament. The ultra-structure of these filaments, determined by cryo-electron tomography, revealed the formation of chimeric viral particles (CVPs) that contained genomes and structural features from both IAV and RSV. Functional assays using a sialidase showed that CVPs can facilitate entry of IAV into cells that were stripped of IAV entry receptors, demonstrating CVPs possess expanded receptor tropism. To determine the likelihood for CVP formation in the airway epithelium, we coinfected hBEC cultures at air-liquid interface. We observed that IAV and RSV infect ciliated epithelial cells and identified foci of coinfected cells. IAV and RSV proteins both localise at the apical surface of coinfected cells, providing opportunity for interactions to occur during viral assembly.

**CONCLUSIONS**

We have characterised a direct interaction between respiratory viruses during viral assembly, which results in formation of a novel class of viral particles. By expanding viral tropism, formation of CVPs may alter viral dissemination within the respiratory tract; potentially impacting disease outcomes for a coinfected individual. Further, by defining a previously unknown source of viral interaction with implications on viral structure, we contribute more widely to the understanding of the properties of IAV and RSV, and their infection biology as a whole.
A new structural approach to study lipid-protein interactions within a viral envelope

**Topic:** Virus structure and replication in influenza virus, RSV and SARS-CoV-2; latest developments in influenza virus, SARS-CoV-2 and RSV molecular virology

**Larisa Kordyukova (1), Petr Konarev (2), Nataliya Fedorova (1), Eleonora Shtykova (2), Aleksander Ksenofontov (1), Oleg Balishchev (3)**

1: Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Russian Federation; 2: Shubnikov Institute of Crystallography of Federal Scientific Research Centre “Crystallography and Photonics” of Russian Academy of Sciences, Moscow, Russian Federation; 3: Frumkin Institute of Physical Chemistry and Electrochemistry, Russian Academy of Sciences, Moscow, Russian Federation

**BACKGROUND**

Influenza A virus, SARS-CoV-2, RSV and other dangerous pathogens belong to various families of enveloped viruses. They contain a lipoprotein envelope surrounding the viral nucleocapsid fulfilling many important functions such as providing nucleocapsid entry into the host cell via fusion of viral and cellular membranes and forming the infectious competent virions. Influenza A virus envelope contains three integral viral proteins: major hemagglutinin, neuraminidase, and minor M2 ion channel. The M1 matrix protein is thought to underlie the lipid bilayer from inside and interact with the lipid component and cytoplasmic domains of integral viral proteins. Lipid-protein interactions which are the basis of the membrane fusion and virus assembly reactions are far from being understood.

**METHODS**

We now suggest an approach to study the physico-chemical mechanisms underlying formation of lipid ordered domains involving HA-M1-lipid interactions during the processes of influenza virus assembly and budding. We used small-angle x-ray scattering (SAXS) and complementary techniques (Electron Microscopy, Dynamic Light Scattering) to analyze the interactions of different components of the viral envelope with M1 matrix protein.

**RESULTS**

Small unilamellar liposomes and proteoliposomes composed of viral lipids extracted from influenza A/Puerto Rico/8/34 (H1N1) virions with or without inserted HA anchoring segments (Figure 1) were incubated with isolated M1 and measured using SAXS.

**CONCLUSIONS**

We conclude that the cytoplasmic tail of Influenza A virus hemagglutinin and membrane lipid composition change the mode of M1 protein association with the lipid bilayer during virus morphogenesis.

This research was financially supported by the Russian Foundation for Basic Research, grant numbers 20-54-12007 (to L.V.) and 20-54-14006 (to O.V.B.)

![Figure 1](attachment:image.png)
The impact of routine molecular point-of-care testing on hospital acquired COVID-19 infection: a pre and post implementation study

**Topic:** Diagnostic testing in the management of acute respiratory infections in primary and secondary care

Tristan Clark (1,2,3), Robert Livingstone (5), Haining Lin (5,6), Brendish Nathan (1,2,3), Paolo Stephen (1,2,3), Tanner Alex (2), Borca Florina (3,4,5), Matthew Stammers (4,5,6)

1: School of Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK;
2: Department of Infection, University Hospital Southampton NHS Foundation Trust, Southampton, UK;
3: NIHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust, Southampton, UK;
4: Clinical Informatics Research Unit, Faculty of Medicine, University of Southampton, Southampton, UK;
5: UHS Digital, University Hospital Southampton NHS Foundation Trust, Southampton, UK;
6: Division B, University Hospital Southampton NHS Foundation Trust, Southampton, United Kingdom

**BACKGROUND**
The risk of hospital acquired COVID-19 (HA-COVID-19) infection is increased by cohorting infected and non-infected patients together in assessment areas whilst awaiting laboratory PCR results. Molecular point-of-care (mPOCT) testing for SARS-CoV-2 has been shown to reduce time to results and improve patient flow but the impact on HA-COVID-19 is unknown.

**METHODS**
In this single centre, pre and post implementation study we analysed the records of all patients admitted and tested for SARS CoV-2 in acute medical admissions areas at University Hospitals Southampton Foundation NHS Trust (UHS). We analysed patients across two time periods; from March 1st to August 13th 2020, prior to the introduction of routine mPOCT and from 14th August 2020 to 1st April 2021, after the introduction of mPOCT. The primary outcome was the proportion of HA-COVID-19 infection, defined as SARS-CoV-2 PCR positivity after 7 days of admission.

**RESULTS**
1988 patients were admitted through the acute medicine admission area and tested for SARS-CoV-2 prior to introducing mPOCT and 4640 were admitted and tested afterwards. The median (IQR) time from admission to SARS-CoV-2 result was 6.5 (2.1-17.9) hours prior to introducing mPOCT and 1.0 (0.8-1.3) hour afterwards (difference of 5.5 hours, 95%CI 8.2 to 18.5; p<0.0001). Median (IQR) length of stay in the assessment cohort area was 12.0 (4.8 to 20.6) hours prior to the introduction of mPOCT and 3.2 (2.0 - 11.9) hours afterwards (difference of 8.8 hours; 95%CI 5.4 to 9.6; p<0.0001). The proportion of HA-COVID-19 cases was 108 (16.9%) of 654 prior to introducing mPOCT and 168 (9.4%) 1782 afterwards. (HR 0.55, 95%CI 0.43-0.70; p<0.0001), despite the circulation of a more infectious variant. Following mPOCT introduction, patients tested with mPOCT were less likely to develop HA-COVID-19 compared with those tested with laboratory PCR, when adjusting of other factors (HR 0.30, 95% CI 0.22-0.41; p<0.0001). Over the study period the weekly median proportion of HA-COVID-19 was low (13.6%, 95%CI 8.2 to 18.9%) compared to other acute trusts within the same region (43.8%, 95%CI 37.8 to 49.9%) and nationally (30.9%, 95%CI 28.4 to 33.5%).

**CONCLUSIONS**
Routine mPOCT for SARS-CoV-2 was associated with a reduced time to results, time spent in admission cohort areas, and in HA-COVID-19 compared to laboratory PCR.

![Figure 1. Median (IQR) time to results (a) and median (IQR) time spent in assessment cohort areas (b)](image-url)
Figure 2. Proportion of patients with hospital acquired COVID-19 before and after introduction of mPOCT.

Figure 3. Median proportion of cases of hospital acquired COVID-19 at UHS, Southern England and across all English NHS trusts over the study period.
Using gargle for monitoring viral shedding in confirmed COVID-19 patients

Topic: Diagnostic testing in the management of acute respiratory infections in primary and secondary care

Nicole Ngai Yung Tsang, Hau Chi So, Benjamin J. Cowling, Gabriel M. Leung, Dennis Kai Ming Ip

WHO Collaborating Centre for Infectious Disease Epidemiology and Control, School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR

BACKGROUND

Gargling has been proposed as a self-sampling swab-free approach for monitoring viral shedding in confirmed COVID-19 patients. With the possibility for self-collection to facilitate SARS-CoV-2 testing, gargle offered an alternative to healthcare-worker-collected nasopharyngeal swab, with no requirement of personal protective equipment and swab availability. However, the testing performance of gargle in RT-PCR testing for monitoring viral shedding remains to be inconclusive in the context of COVID-19. A systematic review and meta-analysis assessing the performance of gargle for monitoring viral shedding in COVID-19 patients is needed.

METHODS

In this meta-analysis, we systematically searched PubMed, Medline, EMBASE, and Web of Science and 2 preprint platforms. We included original clinical studies that examined the performance of gargle and oropharyngeal-nasopharyngeal swab for the monitoring viral shedding among confirmed COVID-19 individuals. Studies without data on testing performance, or those that only examined virucidal effect of gargle were not useful for examining testing performance of a test and were excluded. Testing performance of RT-PCR was examined using random effects models with double arcsine transformation.

RESULTS

A total of 9 studies including 363 confirmed COVID-19 patients were included in our analysis. Using oropharyngeal-nasopharyngeal swabs as the reference comparator, the testing performance of gargle for monitoring viral shedding of SARS-CoV-2 RT-PCR included a sensitivity of 92% (95% CI 75-100), a specificity of 93% (95% CI 73-100), a PPV of 90% (95% CI 59-100), and an NPV of 86% (95% CI 62-100). Performance indicators of gargles were in general heterogeneous.

CONCLUSIONS

To our knowledge, this is the first review systematically assessing the performance of gargling in SARS-CoV-2 RT-PCR testing to inform clinical practice. Our review suggests that, gargle is a reliable, non-invasive, alternative self-sampling approach to nasopharyngeal swabs, for monitoring viral shedding among SARS-CoV-2 confirmed patients. In occasion where swab materials, personal protective equipment and manpower are in short supply, gargling offered scalable capacity for frequent and easily arrangeable SARS-CoV-2 testing for virologic monitoring among COVID-19 patients and to inform patients discharge from hospitals and isolation facilities in resource-limited and remote settings in a more efficient manner.
IFIT27 transcription as a prognostic biomarker for COVID-19

Background

Since the beginning of the COVID-19 pandemic there has been significant interest in developing robust biomarkers to predict disease outcome amongst COVID-19 patients. This facilitates both patient triage and resource prioritisation. Numerous different prognostic biomarkers have been proposed for COVID-19. However, at present there is no consensus on the best diagnostic test to use to predict the outcomes of patients with COVID-19. Moreover, there is no indication as to whether such diagnostic tools would be applicable to other potentially pandemic pathogens and therefore of use to stockpile as part of strategic pandemic preparedness.

Methods

We have previously shown that the transcription of the Interferon alpha-inducible protein 27 (IFIT27) in the blood can be used to discriminate patients with influenza from those with a bacterial infection. Here, we investigate the prognostic capacity of IFIT27 in the blood of an international cohort COVID-19 patients.

Results

Here, we show that IFIT27 is expressed in the respiratory tract of COVID-19 patients and is associated with the presence of a high viral load. We further demonstrate, across multiple patient cohorts, that IFIT27 expression in the blood is associated with COVID-19 severity. Indeed, IFIT27 expression displays a high positive (0.83) and negative (0.55) predictive value and outperforms other more traditional predictors of COVID-19 severity. Importantly, IFIT27 is upregulated in the blood in response to multiple viral pathogens. Moreover, the expression of IFIT27-like genes in the blood is associated with influenza severity.

Conclusion

Together, these data suggest that diagnostic tools targeting this family of genes is likely to be of prognostic use in future viral pandemics, independent of whether they are caused by a coronavirus, an influenza virus or another as yet to be discovered viral pathogen.
Sequential delivery of LAIV and SARS-CoV-2 in the ferret model can reduce SARS-CoV-2 shedding and does not result in enhanced lung pathology.

**Topic:** Co-infections in influenza, RSV disease and COVID-19

Kathryn A. Ryan (2), Katarzyna Schewe (1), Jonathan Crowe (1), Susan A. Fotheringham (2), Yper Hall (2), Richard Humphreys (2), Anthony C. Marriott (2), Jemma Paterson (2), Emma Rayner (2), Francisco J. Salguero (2), Robert J. Watson (2), Catherine J. Whittaker (2), Miles W. Carroll (2,3), Oliver Dibben (1)

1: Flu-BPD, BioPharmaceutical Development, R&D, AstraZeneca, Liverpool, United Kingdom; 2: National Infection Service, Public Health England (PHE), Porton Down, Salisbury, Wiltshire, United Kingdom; 3: Nuffield Department of Medicine, Oxford University, Oxford, United Kingdom

**BACKGROUND**

Co-circulation of SARS-CoV-2 and influenza viruses has been associated with more severe disease outcomes in co-infected patients. In the near-term, influenza vaccination remains of paramount importance in mitigating the risks associated with co-circulation of these viruses, particularly for populations not covered by COVID-19 vaccination programmes.

Live Attenuated Influenza Vaccine (LAIV) is an important tool in protecting against influenza, particularly in pediatric populations. Due to the replication-competent nature of LAIV and its intranasal delivery, co-incident LAIV and SARS-CoV-2 infection is possible. However, it is unknown whether LAIV might influence the outcomes of acute SARS-CoV-2 infection or disease when administrated to children of unknown SARS-CoV-2 status.

Ferrets have been shown to provide a model of LAIV effectiveness as well as a model of mild COVID-19 disease, as often seen in pediatric populations. Here we used ferrets to investigate the effects of LAIV delivery when given in close proximity to SARS-CoV-2 infection.

**METHODS**

Four groups of sixteen ferrets received: SARS-CoV-2 only; quadrivalent LAIV (QLAIV) only; or sequential QLAIV and SARS-CoV-2 infection. In co-infected groups, QLAIV was administered three days pre- or post-SARS-CoV-2 infection to ensure active replication of the first viral inoculum by the time of administration of the second. To determine the impact of QLAIV on SARS-CoV-2 infection and disease, primary endpoints were: the histopathological examination of lung and nasal cavity tissues six days post-SARS-CoV-2 challenge, and the quantification of SARS-CoV-2 viral load in throat swabs at days 1-6 post challenge via RT-qPCR and in lung and nasal cavity tissues at day six post challenge via RT-qPCR and RNAScope.

**RESULTS**

SARS-CoV-2 only control animals showed mild histopathology changes in nasal cavity and, to a lower degree, in lung tissues. Viral RNA was not detected in the lungs of SARS-CoV-2 only control ferrets but showed detectable SARS-CoV-2 viral load in the nasal cavity and shedding in throat swabs, consistent with previous observations. Histopathological examination of co-infected ferrets showed no increase in SARS-CoV-2 lung pathogenesis relative to controls, and only a mild increase in the nasal cavity of co-infected animals. SARS-CoV-2 viral shedding in throat swabs was unchanged in animals receiving LAIV post-SARS-CoV-2 but was significantly reduced in animals receiving LAIV before SARS-CoV-2. This aligned with reductions in SARS-CoV-2 RNA levels in nasal cavity tissues.

**CONCLUSIONS**

LAIV delivery did not exacerbate mild SARS-CoV-2 infection or lung pathology in ferrets, irrespective of the order of administration. Additionally, LAIV administered prior to SARS-CoV-2 infection significantly reduced SARS-CoV-2 replication and shedding in the upper respiratory tract. This work supports the administration of LAIV to children of unknown SARS-CoV-2 status and suggests a potential additional benefit of LAIV administration during the COVID-19 pandemic.
Legionella pneumophila affects the influenza virus-activating host cell protease-repertoire in human cells via two different mechanisms

**Topic:** Co-infections in influenza, RSV disease and COVID-19

**Marie Schwerdtner** (1), Annika Skalik (1), Anna-Lena Jung (2), Jens Dorna (3), Andreas Kaufmann (3), Stefan Bauer (3), Antje Flieger (4), Bernd Schmeck (2), Eva Böttcher-Friebertshäuser (1)

1: Institute of Virology, Philipps-University Marburg, Marburg, Germany; 2: Institute for Lung Research, Universities of Giessen and Marburg Lung Center; Philipps-University Marburg, Marburg, Germany; 3: Institute for Immunology, Philipps-University Marburg, Marburg, Germany; 4: Robert Koch-Institute, Division of Enteropathogenic Bacteria and Legionella/National Reference Centre for Salmonella and other Bacterial Enteric Pathogens, Wernigerode, Germany

**BACKGROUND**

Bacterial co-infection is one of the major risk factors for severe complications during influenza A virus (IAV) infections in humans. Not only is the virus-damaged airway tissue more prone to super-infection by respiratory bacteria such as *Staphylococcus aureus* or *Streptococcus pneumoniae*, but the bacteria themselves also contribute to enhanced viral infection and replication. Therefore, it is important to improve the understanding of viral-bacterial interactions. *Legionella pneumophila*, the causative agent of the Legionnaires’ disease, was identified as a co-infecting agent in IAV infections during the H1N1 pandemic in 2009. However, no previous studies have looked into potential contributions of the bacterium on IAV virulence. Thus, we aimed to investigate whether *L. pneumophila* affects hemagglutinin (HA)-cleavage of IAV, and to identify underlying mechanisms as well as bacterial factors involved.

**METHODS**

In this study, we used two mutants of *L. pneumophila* each lacking one major component of the bacterium: i) Δfla deficient in the major structural component of its flagellum, flagellin, and ii) ΔproA lacking the secreted metalloproteinase ProA.

**RESULTS**

Interestingly, we found that the virus-activating protease TMPRSS2 was significantly upregulated on the mRNA-level in Calu-3 human airway epithelial cells upon stimulation with *L. pneumophila* wildtype but not Δfla, identifying flagellin as the responsible factor. The effect was observed exclusively for TMPRSS2, as other closely related serine proteases were not affected. Enhanced HA-activation in the presence of flagellin is currently under investigation.

Furthermore, we observed that the bacterial metalloprotease ProA facilitated HA-cleavage and viral spread of IAV in HeLa cells. However, ProA did not process HA directly. Instead, we identified the membrane-bound serine protease testisin as hitherto unknown HA-activating host protease mediating HA-cleavage in a ProA-dependent manner. Testisin-mRNA was found to be expressed in alveolar macrophages and primary human bronchial epithelial cells, emphasizing a potential role of this mechanism in vivo.

**CONCLUSION**

Our data demonstrate that *L. pneumophila* influences the protease-repertoire of viral host cells *in vitro* via two distinct mechanisms. The data indicate that protease inhibitors may provide a useful approach for the treatment of influenza and bacterial co-infections to decrease the risk for severe complications.
SARS-CoV-2 replication is inhibited by the presence of co-infecting respiratory viruses in differentiated human airway epithelial cells.

**Topic:** Co-infections in influenza, RSV disease and COVID-19

Kieran Dee, Joanne Haney, Verena Schultz, Pablo Murcia

Centre for Virus Research, University of Glasgow, United Kingdom

**BACKGROUND**

There is strong evidence that co-circulating viruses which cause respiratory disease can negatively impact upon one another at the population and within-host level. Rhinovirus has been shown to trigger an antiviral state in the respiratory epithelium that negatively impacts both influenza A virus (IAV) and SARS-CoV-2 replication. Here, we investigated the effect of other respiratory viruses on SARS-CoV-2 replication in air-liquid interface cultures of bronchial cells.

**METHODS**

Primary bronchial cells were differentiated into pseudostratified cultures recapitulating human airway epithelia. These cultures were infected either singly with SARS-CoV-2 or co-infected with either influenza A virus (IAV) or respiratory syncytial virus (RSV). Staggered infections were also performed by initially infecting with SARS-CoV-2, followed by challenge with either IAV or RSV at 24 and 72 hours post SARS-CoV-2 infection.

**RESULTS**

We found that both IAV and RSV co-infections inhibited SARS-CoV-2 replication. We report that the extent of this inhibitory phenotype is determined by the timings of SARS-CoV-2 infection with respect to the other infecting viruses. We found that both IAV and RSV were able to induce a strong innate immune response in the airway cultures compared to SARS-CoV-2 and that inhibition of SARS-CoV-2 by either virus is dependent on innate antiviral signaling.

**CONCLUSIONS**

IAV and RSV infection can inhibit SARS-CoV-2 replication. Our results indicate that SARS-CoV-2 is highly sensitive to the antiviral state induced by other respiratory viruses in the airway epithelium. In addition, we show that the level of inhibition is likely to be a function of the timing of each individual infection (i.e., the closer the two individual infections occur in time, the more sensitive SARS-CoV-2 is). At a broader scale, these data suggest that SARS-CoV-2 can be negatively impacted by common circulating respiratory viruses and this may shape the future epidemiology of COVID-19.
Viral co-infections in hospitalised COVID-19 patients recruited to the ISARIC WHO CCP-UK study in Scotland

Topic: Co-infections in influenza, RSV disease and COVID-19

Elen Vink (1), Chris Davis (1), Sarah E McDonald (1), Rory Gunson (2), Alasdair Maclean (2), Peter JM Openshaw (3), J Kenneth Baillie (4), Malcolm G Semple (5,6), Antonia Ho (1)

1: Medical Research Council—University of Glasgow Centre for Virus Research, University of Glasgow, Glasgow, United Kingdom;
2: West of Scotland Specialist Virology Centre, NHS Greater Glasgow and Clyde, Glasgow, UK;
3: National Heart and Lung Institute, Imperial College London, London, UK;
4: Roslin Institute, University of Edinburgh, Easter Bush, Edinburgh, UK;
5: Health Protection Research Unit in Emerging and Zoonotic Infections, Institute of Infection, Veterinary and Ecological Sciences, Faculty of Health and Life Sciences, University of Liverpool, Liverpool, UK;
6: Department of Respiratory Medicine, Alder Hey Children’s Hospital, Liverpool, UK

BACKGROUND

The prevalence of respiratory viral co-infection in patients with COVID-19 and its impact on disease severity is unclear, due to a lack of systematic testing in addition to a bias towards testing patients requiring critical care. We aim to assess the prevalence of viral co-infection in a well characterised cohort of hospitalised COVID-19 patients in Scotland, and to investigate the impact of co-infection on disease severity and clinical outcomes.

METHODS

A validated in-house multiplex real-time polymerase chain reaction (PCR) test for respiratory viruses, developed at the West of Scotland Specialist Virology Centre, was performed on upper respiratory tract samples (URT) from hospitalised COVID-19 patients recruited to the International Severe Acute Respiratory and Emerging Infections Consortium (ISARIC) WHO Clinical Characterisation Protocol UK (CCP-UK) study in Scotland. URT samples collected at recruitment were tested for adenovirus, rhinovirus, respiratory syncytial viruses (RSV) A and B, influenza A and B viruses, parainfluenza viruses 1-4, human coronaviruses NL63, 229E and OC43, and human metapneumovirus. Cycle threshold of <40 was considered positive. Comprehensive demographic, clinical and outcome data were collected prospectively up to 28 days post discharge. Disease severity was categorised based on the patients’ maximum level of respiratory support, and clinical outcome. Patients with URT samples obtained >14 days from admission and those with nosocomial COVID-19 infection were excluded.

RESULTS

Multiplex PCR was performed on URT samples from 306 patients admitted to 8 Scottish hospitals between 4th March and 14th October 2020. The median patient age was 60 years (IQR 50-72) and 60.5% of patients were male. Median duration from symptom onset to sample collection was 9 days (IQR 6-13). 54 (17.6%) patients required mechanical ventilation and 40 (13.1%) died in hospital.

A co-infecting respiratory virus was detected in 9 (2.9%) COVID-19 patients. Rhinovirus and Parainfluenza virus 4 were identified in 3 (1%) and 2 (0.7%) patients, respectively, while Influenza B virus, human coronavirus NL63, and parainfluenza viruses 2 and 3 were each detected in 1 patient. Patients with viral co-infection were all recruited prior to 7th April 2020. The low prevalence of viral co-infections precluded analysis of association with disease severity.

CONCLUSION

A low prevalence of viral co-infection was observed among hospitalised COVID-19 patients in Scotland during the first and early part of the second wave of the pandemic. The study period was outside of normal seasonal transmission of respiratory viruses. Furthermore, the absence of co-infecting viruses beyond early April may also be due to the widespread implementation of non-pharmacological interventions, such as physical distancing, mask wearing and school closures. With the relaxation of these measures, it will be important to monitor the prevalence and impact of viral co-infections and interactions in the forthcoming winter.
HLA-E-dependent antigen-specific memory NK cell responses develop against influenza nucleoprotein and are cross-reactive between antigendically distinct strains

Topic: Innate and adaptive immunity towards influenza, RSV disease and COVID-19

Olivier Lucar (1), Taylor Yoder (1), Sho Sugawara (1,2), Alexandra Werner (1), Joshua Ghefran (1), R. Keith Reeves (1,2), Stephanie Jost (1,2)

1: Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School, United States of America; 2: Division of Infection and Comparative Immunology, Center for Human Systems Immunology, Duke University School of Medicine, United States of America

BACKGROUND
Mobilization of immune effector cells with potent antiviral activity against conserved influenza antigens represents one approach to achieve vaccine-elicited long-lasting protection against a broad range of influenza strains. Natural killer (NK) cells are classically considered part of the innate immune system based on their ability to mediate rapid and nonspecific responses playing critical roles in defense against viral infections, including influenza. For over a decade, studies have demonstrated that beyond their ability to eliminate infected cells without the need for prior sensitization, NK cells are also capable of antigen-specific immunological memory and may thus represent a third arm of the immune system that can be targeted by vaccines. In particular, exposure to influenza antigens induces protective influenza-specific memory NK cells in mice, yet clear evidence of influenza-specific memory NK cells in humans is still lacking.

METHODS
Primary NK cells and clonally expanded single NK cells (NKCL) from healthy donors were used to measure influenza-specific NK cell responses against peptide pools derived from the nucleoprotein (NP) of H1N1, H2N2 or H3N2 strains using intracellular cytokine staining and cytototoxicity assays. NKCL/NK cell responses to different influenza strains were assessed after co-culture with autologous BLCL pulsed with NP- or self-derived peptide pools. The ability of NP-derived peptides to stabilize HLA-E expression on K562 cells stably transduced with HLA-E*0101 was determined by flow cytometry and HLA-E-stabilizing peptides further evaluated for their capacity to mediate antigen-specific NK cell killing.

RESULTS
NK cells from 45% of adults were found to mediate robust responses against conserved antigens from at least 2 heterosubtypic influenza strains completely independent of T cell help. To determine if single influenza-specific NK cells can efficiently respond to NP from distinct strains, we measured H1N1, H2N2 and H3N2 NP-specific killing by 24 NKCL from 8 donors. 25% of NKCL showed over 15% specific killing against influenza NP (17%-100% specific killing), among which 43% robustly reacted against both H1N1 and H3N2 strains (23%-100% specific killing). 16% of all tested NKCL displayed heterosubtypic cytotoxicity against H1N1, H2N2 and H3N2 strains. Receptor blockade experiments have shown that in primate species antigen-specific memory NK cell responses are largely dependent on expression of the activating NKG2C receptor, a ligand for HLA-E, and potent influenza-specific killing by NKCL was associated with high NKG2C expression. Accordingly, we identified single HLA-E-binding NP-derived peptides that trigger dominant responses by influenza-specific memory NK cells.

CONCLUSIONS
Collectively, these findings suggest that vaccine strategies that also enhance the breadth of influenza-specific NK cell responses could significantly improve vaccine-induced cross-protection.
**Effects of metabolic disease on humoral immunity to SARS-CoV-2 infection**

*Topic: Innate and adaptive immunity towards influenza, RSV disease and COVID-19*

**Background**

COVID-19 is an ongoing worldwide pandemic, caused by SARS-CoV-2. Metabolic conditions (diabetes mellitus and/or obesity) increase the severity of SARS-CoV-2 infection. However, what effect these metabolic conditions have on the longevity of the antibody response to SARS-CoV-2 remains unclear. These data are essential in understanding the susceptibility of patients with metabolic conditions to re-infection as well as the necessity for vaccination in this population.

**Methods**

Patients with a previous clinical history of a positive SARS-CoV-2 PCR have been recruited from various locations in Australia. The following clinical information were obtained from recruited patients: age; sex; diabetes status (type 1 vs type 2 vs none); HbA1c (i.e. blood glucose level); current medications; other co-morbidities (chronic respiratory disease, obesity, cardiovascular disease and/or hypertension); date of positive SARS-CoV-2 PCR test & clinical data at time of positive PCR test (disease symptoms, hospital and/or ICU admission and duration). Serum samples were collected from recruited patients.

Sera collected from convalescent patients were used on 2 different assays: ELISAs and microneutralization assays. Antibody titres (IgG) were determined by running SARS-CoV-2 Spike and Nucleocapsid protein ELISAs. Neutralization activities were determined by microneutralization assays with the original SARS-CoV-2 virus and the Delta variant.

**Results**

Our data shows that patients who suffered severe disease course have higher antibody titres 3-6 months post recovery. COVID-19 recovered patients with diabetes mellitus also had higher antibody titres and neutralization activity as compared to healthy recovered patients. Antibody longevity is defined by the change of both neutralization activity and antibody titres over time. Our data shows that COVID-19 recovered patients living with diabetes mellitus have decreased antibody longevity than healthy recovered patients. Similarly, the same trend is observed in patients with increased body mass index.

**Conclusions**

We have provided evidence of a shorter-lived circulating antibody titres in patients with chronic metabolic disease. Prioritising vaccination in this population, even amongst convalescent individuals, is of paramount importance.
DEVELOPMENT OF EXPERIMENTAL LIVE INFLUENZA VACCINE STRAINS WITH MODIFIED NP AND NS GENES

**Topic:** Innate and adaptive immunity towards influenza, RSV disease and COVID-19

Polina Prokopenko, Victoria Matyushenko, Arina Goshina, Anna Chistyakova, Daria Mezhenskaya, Ivan Sychev, Ekaterina Zimmerman, Irina Isakova-Sivak, Larisa Rudenko

Institute of Experimental Medicine, Russian Federation

**BACKGROUND**

The effectiveness of licensed seasonal influenza vaccines remains suboptimal. To improve the efficacy of LAIV several approaches have been explored: (i) truncating the NS open reading frame to enhance innate and adaptive immune responses; (ii) incorporating nucleoprotein (NP) from the wild-type parental virus into the LAIV genome (i.e., replacing the genome formula from 6:2 to 5:3), to optimize the T-cell response.

**METHODS**

Mutagenesis of the NS gene of master donor virus A/Leningrad/134/17/57 (Len/17) was performed to shorten the NS protein to 126 a.a. Recombinant LAIV viruses carrying HA and NA genes of A/Anhui/1/2013 (H7N9) virus were rescued by electroporating Vero cells with corresponding set of plasmids. Viruses were grown in eggs at 33°C, and their ts/ca phenotypes were studied by incubating infected eggs at 38°C and 26°C, respectively. C57BL/6J mice were immunized intranasally at a dose of 6 lg EID$_{50}$, twice at 21-days intervals. On day 3 after immunization, infectious virus titers were determined in nasal turbinates and lungs by titration of tissue homogenates in eggs. Serum samples were collected three weeks after the second immunization, and IgG antibody levels to the whole H7N9 6:2 rg virus were determined by ELISA. T-cell responses were assessed by stimulating mouse splenocytes collected on day 7 after the second dose with NP peptides.

**RESULTS**

LAIVs H7N9 with 6:2 and 5:3 genome compositions expressing truncated NS1-126 were generated by the means of reverse genetics. All LAIV viruses were restricted in replication at elevated temperatures of 38°C (its phenotype), whereas they retained the cold-adapted phenotype, regardless of the genome formula. All viruses lacked the ability to replicate in the mouse lungs, indicating the attenuated phenotype. Only viruses with the full-length NS1 proteins replicated in the upper respiratory tract of mice after intranasal administration. The two-dose immunization of mice resulted in high IgG antibody levels in all vaccine groups, with enhanced antibody production in the NS-126 group. T-cell responses specific to the NP366 epitope present in the recent H7N9 virus were higher in the LAIV 5:3 group relative to the LAIV 6:2 group, suggesting that the replacement of the old MDV NP protein by the recent one will result in more optimal cellular immunity after vaccination.

**CONCLUSIONS**

Modifications of LAIVs did not impair the replicative properties of the vaccine virus in vitro. Truncation of NS1 protein can significantly increase the immunogenicity of the live attenuated vaccine. Furthermore, the truncation of NS1 protein leads to the reduced viral replication in the respiratory tract of mice, indicating a more attenuated phenotype of the virus compared to the strains with full NS1. These data suggest that modified vaccines might have the increased safety profile and can be recommended for use in children younger than three years of age.

**Funding:** this study was funded by the Russian Federation President grant MD-327-2020-7.
T-cell immune responses to live SARS-CoV-2 after natural infection and vaccination

**Topic:** Innate and adaptive immunity towards influenza, RSV disease and COVID-19

Irina Isakova-Sivak, Victoria Matyushenko, Igor Kudriavtsev, Ekaterina Stepanova, Arina Goshina, Anna Chistyakova, Polina Prokopenko, Ivan Sychev, Larisa Rudenko

**Institute of Experimental Medicine, Russian Federation**

**BACKGROUND**

Assessment of the levels of antiviral immunity in patients who recovered from the new coronavirus infection, as well as in those vaccinated with COVID-19 vaccines, is necessary to monitor the immunological status of the population and the effectiveness of vaccines used in practice. Most often, the immune responses are measured by the levels of serum antibodies that bind to the spike protein of the SARS-CoV-2 virus, or are able to neutralize the live virus in in vitro experiments. The levels of IgG and virus-neutralizing antibodies decrease significantly within 6-9 months after illness or after immunization, however, the real protective potential of the immune system may be maintained by the long-lived memory B and T cells. In this study, we developed assays to identify key subpopulations of memory T cells specific to the SARS-CoV-2 virus in COVID-19 convalescents and people vaccinated with the Sputnik V vaccine.

**METHODS**

Fifty COVID-19 convalescents and thirty subjects immunized with Sputnik V donated whole blood at different periods post-infection or vaccination. All participants signed an informed consent before sample collection. Peripheral blood mononuclear cells (PBMCs) were isolated using standard protocol and stimulated with live, sucrose gradient purified SARS-CoV-2 virus in a BSL-3 laboratory. Several SARS-CoV-2 doses were assessed to find an optimal dose for PBMC stimulation. As a biological control, stimulation with H1N1 influenza virus was used. Intracellular cytokine staining (ICS) assay was used to count IFNγ-producing CD4 and CD8 effector memory T (Tem) cells.

**RESULTS**

An optimal dose of SARS-CoV-2 for stimulating PBMCs of COVID-19 recovered subjects was found to be 0.001 MOI, which is significantly lower than the dose of an influenza virus in this assay (MOI=1). Both natural infection and vaccination induced virus-specific T-cell responses. Stimulation of PBMCs with live SARS-CoV-2 revealed IFNγ-producing Tem cells with CD4+CD45RA-CCR7- phenotype, which persisted in circulation for up to 12 months after COVID-19, whereas cytotoxic effector memory T cells were found at significant levels only shortly after the disease, but rapidly declined over time. Moderate levels of SARS-CoV-2-specific CD4 and CD8 Tem cells were also found in vaccinated individuals, although these levels significantly decreased during 6 months after vaccine administration.

**CONCLUSIONS**

Stimulation of PBMCs with live SARS-CoV-2 can provide important information on the ability of the immune system to recognize all viral antigens and protect an individual from natural infection. Stimulation of immune cells with live SARS-CoV-2 revealed a rapid decline in the pool of effector memory CD8+, but not CD4+, T cells after recovery from COVID-19. Both Tem subsets were induced by Sputnik V vaccine, although these responses were not long-lived. These data provide additional information on the development and persistence of cellular immune responses after natural infection and vaccination, and can inform further development of T cell-based SARS-CoV-2 vaccines.

**Funding** The study was funded by the RSCF grant №21-75-30003.
Identified Pregnancies and Associated Outcomes Across the Novavax COVID-19 Vaccine Clinical Development Program

**Topic:** Lessons learned from and prospects for COVID-19 vaccination

Hadi Beyhaghi, Anthony Marchese, Crosby Winters, Steve Gao, Katherine Smith, Seth Toback

Novavax Inc. United States of America

**BACKGROUND**

Limited data exist on the outcomes of identified pregnancies among the participants of COVID-19 vaccines clinical trials. This analysis aimed to identify and characterize pregnancies among participants in the Novavax COVID-19 vaccine (NVX-CoV2373) clinical development program.

**METHODS**

We identified all pregnancies among female participants across the Novavax COVID-19 vaccine clinical development program, including a phase 1/2 trial in the US/Australia, a phase 2b trial in South Africa, and two phase 3 trials in the UK and US/Mexico. For each identified pregnancy as of 3 August 2021, age, country, dates of administration of blinded doses of vaccine or placebo, last menstrual period (LMP) and positive pregnancy test dates, and pregnancy outcomes with corresponding dates were extracted and described. Relative frequency of pregnancy outcomes (number of each outcome divided by the total number of pregnancies) is summarized as percentages and continuous measures are reported as median (interquartile range) or mean (standard deviation).

**RESULTS**

A total of 108 pregnant participants with mean age (standard deviation) 29.8 (6.5) years were identified across the Novavax COVID-19 vaccine clinical development program, including 2 pregnancies from the phase 1/2 trial in the US/Australia (2%), 17 pregnancies from the phase 3 trial in the UK (16%), 28 pregnancies from the phase 3 trial in the US/Mexico (26%), and 61 pregnancies from the phase 2b trial in South Africa (56%). Among these pregnancies, the following outcomes occurred: 9 live births (8%), 11 voluntary terminations (10%), 16 spontaneous abortions (15%), one case of ectopic pregnancy (1%), 7 pregnancies with unknown outcome status (6%), and 64 ongoing pregnancies (59%). No other birth outcomes were available. The median (interquartile range) follow-up period was 19 (12-32) weeks. The table below presents the frequency of pregnancy outcomes by timing of the first blinded study dose relative to the LMP.

**CONCLUSIONS**

This analysis provides preliminary descriptive data on identified pregnancies and the associated outcomes across the Novavax COVID-19 vaccine clinical development program. Continued follow up of ongoing pregnancies and future analysis of unblinded data can further inform the safety profile of NVX-CoV2373.

<table>
<thead>
<tr>
<th>Pregnancy Outcome</th>
<th>First study dose timing relative to LMP</th>
<th>Total (n=108)</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prior Gestation /10 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live Birth</td>
<td>0</td>
<td>1</td>
<td>9 (8%)</td>
</tr>
<tr>
<td>Spontaneous Abortion</td>
<td>10</td>
<td>1</td>
<td>10 (9%)</td>
</tr>
<tr>
<td>Voluntary Termination</td>
<td>5</td>
<td>1</td>
<td>11 (10%)</td>
</tr>
<tr>
<td>Ectopic Pregnancy</td>
<td>4</td>
<td>1</td>
<td>4 (4%)</td>
</tr>
<tr>
<td>Ongoing or Unknown</td>
<td>47</td>
<td>3</td>
<td>73 (68%)</td>
</tr>
</tbody>
</table>

Table: Frequency of pregnancy outcomes by timing of first blinded study dose relative to LMP
Nanoemulsion adjuvanted SARS-CoV-2 vaccine induces systemic and mucosal immune response following intranasal vaccination

**Topic:** Lessons learned from and prospects for COVID-19 vaccination

**Margaret Lugin** (1), Shyamala Ganesan (1), Xiaomei Ge (2), Dan Luo (2), Brian Zabel (2), Ali Fattom (1)

1. BlueWillow Biologics, Ann Arbor, MI, United States of America; 2. Lake Pharma, Now Part of Curia, San Carlos, CA, United States of America

**BACKGROUND**

Intramuscular COVID-19 vaccines are effective in preventing COVID-19 disease, but do not induce sterilizing immunity, as they do not protect against upper respiratory tract colonization. Booster vaccinations are likely needed for previously vaccinated populations due to waning immunity and the prevalence of the highly transmissible delta variant that is leading to an increase in breakthrough infections. Intranasal vaccines against COVID-19 can complement the intramuscular vaccines and offer potential for sterilizing immunity, with induction of mucosal immunity in the upper and lower respiratory tract, in addition to boosting systemic immunity. Sterilizing immunity could reduce the transmission of SARS-CoV-2 as the virus will be neutralized in the nasal passages, thereby limiting the spread of the virus. Nanoemulsion adjuvanted SARS-CoV-2 vaccine delivered intranasally can facilitate mass vaccinations using easy and quick needle free administration, with added advantage of thermostability. In this study, two SARS-CoV-2 antigens were formulated with nanoemulsion adjuvant for intranasal delivery in mice to induce both mucosal and systemic immunity against SARS-CoV-2.

**METHODS**

Two vaccine candidates were tested for induction of immune response to SARS-CoV-2 in CD-1 mice. The LP-438, a monomeric receptor binding domain (RBD) protein, or LP-635, a multimeric RBD in nanoparticle form, were formulated with the nanoemulsion (NE01). Mice (n=8) received two or three doses of either vaccine, three weeks apart. Mice were sacrificed 2 weeks after their final immunization and plasma, spleens, and lungs were collected. Plasma was analyzed for presence of systemic immune response along with neutralizing antibody titers. Tissues were processed for homing IgG and IgA memory B cell, and Th1, Th2, and Th17 cytokines.

**RESULTS**

LP-438/NE01 and LP-635/NE01 were well tolerated in mice. Plasma from vaccinated animals demonstrated significant induction of systemic IgG and IgA antibodies with neutralizing activity against SARS-CoV-2 spike or ACE2 protein. Additionally, the multimeric LP-635/NE01 immunized animals induced a robust systemic response following 2 immunizations, compared to LP-438/NE01, where robust response was only observed in half of immunized mice, even after 3 immunizations. A balanced Th1/Th2 immune response with significant induction of Th17 in lungs and spleens, and significant levels of antigen specific IgG and IgA memory B cells was seen in LP-635/NE01 immunized mice, but not in LP-438/NE01 immunized mice.

**CONCLUSIONS**

LP-635/NE01 is a promising COVID-19 intranasal vaccine candidate, capable of inducing both antigen-specific mucosal and systemic immunity to SARS-CoV-2.
Lessons learned from and prospects for COVID-19 vaccination

**Topic**: Lessons learned from and prospects for COVID-19 vaccination

Paul Van Buynder (1), Angela Newbound (2), Raina MacIntyre (3), Alexander Kennedy (4), Chris Clarke (4), Jonathan Anderson (4)

1: Department of Medicine, Griffith University, Southport, Queensland, Australia; 
2: Independent Consultant, Semaphore, SA, Australia; 
3: Biosecurity Research Program, The Kirby Institute, University of New South Wales, Sydney, NSW, Australia; 
4: Seqirus Pty Ltd, Melbourne, VIC, Australia

**BACKGROUND**

COVID-19 and Influenza vaccination programs are likely to co-exist for many seasons to come. In 2021, Australia experienced its first concurrent COVID-19 and flu vaccine program. Here we draw out learnings for maximising immunisation uptake.

**METHODS**

We reviewed the events leading up to and during the 2021 COVID-19 and Influenza vaccine programs in Australia (cut-off at June 30 2021). Learnings were developed based on author experience.

**RESULTS**

The COVID-19 vaccine program commenced on February 21st and initially progressed slowly, achieving 17% of the 4 million dose target for March 30th. The delays were largely due to unexpected constraints in offshore AstraZeneca (AZ) vaccine supply as well as the logistical challenges of -80 °C Pfizer vaccine storage. The delays meant a focus on flu vaccination was deferred despite influenza vaccines being available, while the differences in settings for COVID-19 and influenza vaccine meant mass clinics could not schedule flu vaccinations and family practices could not use recall systems for targeted COVID-19 vaccination. Future programs should learn from these challenges and integrate the two separate programs into one program co-ordinated by experienced public health experts. This co-ordination could maximise the channels through which both vaccines can be booked and administered, plan eligible patient recall for one vaccine ahead of their eligibility for the other, release timely supply and communications expanding the window for vaccination, and design sequencing that accounts for and minimises exclusion windows to ensure patients receive all recommended vaccines.

Plans for the locally manufactured AZ vaccine, made by CSL/Seqirus at a rate of 1 million doses per week, to resolve the early supply challenges were complicated by the rare thrombosis with thrombocytopenia events which received widespread media attention in an environment of negligible COVID-19 transmission. The resultant mixed messaging from stakeholders, repeated changes to COVID-19 vaccine recommendations, and the limited and delayed communication from Government about influenza vaccination caused challenges. These are a reminder that efficient program delivery requires that Government and other stakeholders align on clear, consistent, and timely communications which highlight the importance and urgency for receiving both vaccinations.

**CONCLUSIONS**

Opportunities to maximise influenza vaccine uptake in Australia for SH21 were missed. To maximise both influenza and COVID-19 vaccine uptake a centralised, co-ordinated campaign informed by policy recommendations and the timing and availability of both influenza and COVID-19 vaccines is required and should be continuously updated. These may offer insight for program managers in other regions or during future seasons.
Heterologous ChAdOx1/BNT162b2 vaccination induces stronger immune response than homologous ChAdOx1 vaccination

**Topic:** Lessons learned from and prospects for COVID-19 vaccination

Zoltan Banki (1), Jose Mateus (2), Annika Schäfer (1), Helena Schäfer (1), David Banle (1), Lydia Riepler (1), Sabine Embacher (3), Alba Grifoni (2), Alessandro Sette (2,4), Viviana Simon (5,6,7,8), Barbara Falkensammer (1), Hanno Ulmer (9), Bianca Neurauter (1), Wegene Borena (1), Florian Krammer (5,6), Dorothee von Lazer (1), Daniela Weiskopf (2), Janine Kimpel (1)

1: Institute of Virology, Medical University of Innsbruck, Austria; 2: Center for Infectious Disease and Vaccine Research, La Jolla Institute for Immunology (LJI), La Jolla, CA 92037, USA; 3: Competence Center for Clinical Trials, Medical University of Innsbruck, Innsbruck, Austria; 4: Department of Medicine, Division of Infectious Diseases and Global Public Health, University of California, San Diego (UCSD), La Jolla, CA 92037, USA; 5: Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA; 6: Department of Pathology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA; 7: Division of Infectious Diseases, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA; 8: The Global Health and Emerging Pathogen Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA; 9: Department of Medical Statistics, Informatics and Health Economics, Medical University of Innsbruck, Austria

**BACKGROUND**

Several COVID-19 vaccines have been approved, with an mRNA vaccine from Pfizer/BioNTech (Comirnaty, BNT162b2, BNT) and a vector vaccine from AstraZeneca (Vaxzevria, ChAdOx1, AZ) being widely used. mRNA vaccines induce high antibody and T cell responses, also to SARS-CoV-2 variants, but are costlier and less stable than the slightly less effective vector vaccines. For vector vaccines, heterologous vaccination schedules have generally proven more effective than homologous schedules. Thus, we tested if heterologous vaccination with AZ followed by BNT may elicit superior immune response to the homologous AZ regimen.

**METHODS**

In the HEVACC 3-arm, single-blinded study, the AZ/AZ and the AZ/BNT arms were randomized, the third arm (BNT/BNT) was observational. We compared the reactogenicity and immunogenicity between the homologous regimens to heterologous vaccination (AZ/BNT).

**RESULTS**

Anti-SARS-CoV-2 spike protein IgG, IgA and neutralizing antibody levels were significantly higher in the AZ/BNT and the BNT/BNT groups than in the AZ/AZ group. This also applies to the neutralizing antibodies against immune escape variants B.1.351 (Beta) and B.1.617.2 (Delta). Similarly, spike-specific T cell responses as measured in three different assays were comparable in the AZ/BNT and the BNT/BNT groups, but significantly lower in the AZ/AZ vaccinees. The proportion of multifunctional T cells was higher in the AZ/BNT group than in the other two groups. No difference in the reactivity of T cells to peptide pools from different immune escape variants was seen.

**CONCLUSIONS**

This study clearly shows the excellent immunogenicity and safety of heterologous AZ/BNT vaccination and encourages further studies on heterologous vaccination schedules.
Long term circulation of Avian Influenza Viruses Subtype H7 in wild birds in the Azov-Black Sea Region and detection of new hosts

**Topic:** Pandemic threats from the animal world

Denys Muzyka (1), Oleksandr Rula (1), Susanne Koethe (2), Anne Günther (2), Jacqueline King (2), Borys Stegniy (1), Anne Pohlmann (2), Mary Pantin-Jackwood (3), Martin Beer (2)

1: National Scientific Center - Institute of Experimental and Clinical Veterinary Medicine, Ukraine; 2: Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany; 3: Southeast Poultry Research Laboratory, USDA, Athens, Georgia, USA

**BACKGROUND**

Wild aquatic birds are the primary reservoirs and carriers of avian influenza viruses (AIVs). AIVs subtype H7 are one of the most dangerous for humans and birds. Outbreaks in poultry caused by the H7 viruses were recorded in different countries: USA (2017-2018, H7N8, H7N9), China (2013 – present, H7N9). Since 2013, 1567 laboratory-confirmed AI/H7N9 human cases have been reported worldwide. In 2018, the new AIV (H7N4) virus infection was detected in humans in China. Study of the influenza virus of this subtype is critically important in the context of identifying potentially dangerous viruses.

**METHODS**

Surveillance for AIV in the Azov Black Sea region of Ukraine (2001-2021) generated samples from 27,646 wild birds of 133 species. Samples were tested by virological methods, sequencing using OIE guidelines. Sequencing was done in Southeast Poultry Research Laboratory, Friedrich-Loeffler-Institute.

**RESULTS**

Ukraine is one of many countries affected by highly pathogenic AIV outbreaks. Four waves of the HPAIV were reported in 2005-2006 and 2008 (H5N1), 2016-2017, and 2020-2021 (H5N8, H5N6). Outbreaks in industrial and backyard poultry of AIV subtype H7 have never been registered in Ukraine. At the same time, antibodies to subtype H7 were detected in 2.3 – 25% of wild birds in 2006-2009. In 2006-2016, seven H7 AIV (H7N3, H7N7) were isolated from fecal of clinically healthy mallard (Anas platyrhynchos). In 2020, two H7 AIV (H7N3, H7N2) were isolated from cloacal samples of clinically healthy mallard (Anas platyrhynchos). During winter season 2021 one H7 AIV was isolated from a new host – Fieldfare (Turdus pilaris). This is the first case of isolation AIV from this species. All isolates were low pathogenic AIVs. Phylogenetic analysis of the HA revealed that all AIVs (H7N3, H7N7) isolated in 2010-2011 showed a 99-52-99.82% nucleotide identity to each other and to the viruses circulated in the Netherlands and Georgia in 2010-2013. AIVs (H7N3) isolated in 2014 had 99.53% nucleotide identity to the AIVs isolated in 2016 in Georgia, and 96.46 - 96.50% to the AIVs in Asia (Korea, Mongolia) and South Africa. AIVs (H7N3) isolated in 2010-2011 were 98-99% nucleotide identity with subtypes H2N3, H4N3, H7N3 from the Netherlands, Georgia in 2008-2012 by the phylogenetic analysis of the NA gene. AIV H7N7 had 97.4 – 99.93% nucleotide identity to the influenza viruses subtype H2N7, H7N7 from Georgia. AIV’s (H7N3, H7N2) isolated in 2020 had 99.75-97.11% nucleotide identity to the AIVs isolated in Georgia and Egypt.

**CONCLUSION**

Results confirmed the special role of the Azov-Black Sea region of Ukraine in maintaining the circulation and wide genetic diversity of viruses (including H7). Also very important are looking for new hosts and resources of AIV in wild nature in this region that should be evaluated as a connecting chain between Asia and Europe.
Adaptive potential of zoonotic avian influenza virus H7N9 to ducks

Topic: Pandemic threats from the animal world

Susanne Koethe, Lorenz Ulrich, Angele Breithaupt, Christian Grund, Anne Pohlmann, Timm Harder, Martin Beer

Friedrich-Loeffler-Institut, Germany

Migratory waterfowl are potential vectors for the long-range transmission of avian influenza viruses (AIV) globally. In 2013, low pathogenic avian influenza viruses (LPAIV) of subtype H7N9 emerged in China and evolved into highly pathogenic avian influenza virus (HPAIV) variants that were first detected in the beginning of 2017. Extensive co-circulation of both pathotypes was particularly restricted to galliform poultry but caused several thousand severe human infections with a high fatality ratio in China. The systematic surveillance revealed constant evolution of AIV H7N9 and reassortment events with the Eurasian wild bird gene pool. Spillover and adaptation of either LPAIV or HPAIV H7N9 from galliform to anseriform poultry into migratory waterfowl would justify severe concerns about a possible transcontinental spread of these highly zoonotic viruses.

The potential of AIV H7N9 viruses to adapt to wild waterfowl was determined using an LPAIV (A/duck/Japan/AQ-HE28-3/2016) and two HPAIV H7N9 isolates, (A/duck/Japan/AQ-HE29-22/2017 (HE29-22), and (A/duck/Japan/AQ-HE29-52/2017 (HE29-52)). Replication competence and transmissibility, respectively, were characterized in embryonated duck eggs, in one-week old ducklings and in four-week old ducks.

The LPAIV strain revealed no pathogenicity and transmissibility in ducks. In contrast to the LPAIV, the two HPAV variants induced systemic infection in duck embryos. When applied intra-muscularly to one-week old ducklings, the HPAIV HE29-22 and HE29-52 isolates caused mortality, indicating increased virulence. In four-week old ducks, oronasal inoculation of both respective HPAIV H7N9 isolates lead to virus shedding and efficient transmission to contact ducks without onset of disease.

The data indicate a significant adaptive potential of HPAIV, but not LPAIV, H7N9 isolates to ducks: Asymptomatic but productive infection in four-week old ducks justifies fears of undetected regional and possibly global virus spread with ducks and migratory anseriform birds, respectively.
Defining the Genesis and Pathogenesis of the Avian Influenza Virus

**Topic:** Pandemic threats from the animal world

Lauren E Steele (1), Anjana Karawite (1), Jane E Sinclair (1), Keng Yih Chew (1), Cassandra Pegg (1), Tambet Teesalu (2), Giuseppe Balistreri (3), Kirsty R Short (1)

1. School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane Australia;
2. Laboratory of Cancer Biology, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia;
3. Faculty of Biological and Environmental Sciences, Molecular and Integrative Biosciences Research Program, University of Helsinki, Helsinki, Finland

**BACKGROUND**

Influenza A viruses can readily spread from wild bird species to terrestrial poultry. In terrestrial poultry, influenza viruses cause a mild/subclinical infection and is thus referred to as low pathogenic avian influenza (LPAI). H5 and H7 LPAI viruses can evolve in terrestrial poultry to become highly pathogenic avian influenza (HPAI) viruses. Unlike LPAI viruses, HPAI viruses can cause 100% mortality in terrestrial poultry. The evolution of LPAI to HPAI is associated with the insertion of a multi-basic cleavage site in the haemagglutinin, which conforms to the Cend rule (CendR). The CendR motif is a short peptide with the sequence R/K-X-X-R/K, which has been experimentally proven to bind to, and be internalised by, neuropilin-1 (NRP-1). Here, we investigate the structure and expression of NRP-1 in several avian cell lines to determine its role in promoting the evolution of LPAI to HPAI in chicken cells.

**METHODS**

To investigate structural differences between avian (chicken and duck) NRP-1, amino acid changes in chicken and duck NRP-1 were introduced into the human NRP-1 protein structure using FoldX to model avian NRP-1 structures. Structural alignments were then conducted using PyMOL to determine structural variations between the two avian species. NRP-1 gene expression was determined by culturing primary aortic endothelial cells isolated from chickens and ducks (ChAEC and DuAEC), before harvesting cellular RNA for quantitative polymerase chain reaction analysis. Protein expression in ChAEC and DuAEC was assessed using fluorescently-labelled NeutrAvidin-coated silver nanoparticles conjugated to a CendR peptide.

**RESULTS**

Structural differences and mRNA expression levels between chicken and duck NRP-1 were minimal. Conversely, NRP-1 protein expression was significantly higher in ChAEC than in DuAEC, suggesting that there may be greater selective pressure for the genesis of HPAI variants in ChAEC due to a greater abundance of NRP-1. These results are also seen in chicken and duck fibroblasts, suggesting this phenotype is not cell-type-specific.

**CONCLUSIONS**

The differential protein expression of NRP-1 in ChAEC and DuAEC may explain why HPAI variants only emerge in terrestrial poultry. Greater levels of NRP-1 at the surface of chicken cells may provide the positive selective pressure for HPAI containing the multi-basic cleavage site. These data may provide the molecular basis for genetically engineering chickens that are resistant to the genesis of HPAI.
Genetic determinants for virulence and adaptation of clade 2.3.4.4 H5N8 viruses in chickens, ducks and mammals

Topic: Pandemic threats from the animal world

Elsayed Abdelwhab, Claudia Blaurock, David Scheibner, Angele Breithaupt, Thomas Mettenleiter
Friedrich Leeffler Institut, Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany

BACKGROUND
Since 1997, avian influenza viruses (AIV) of H5N8 goose/Guangdong lineage continue to cause severe losses in poultry and wild birds and pose a serious pandemic risk. This H5Nx lineage exhibited high flexibility for mutations resulting in tens of clades/subclades and undergone several reassortment events. From 2014 to 2021, the panzootic H5N8 clade 2.3.4.4 AIV spread on an unprecedented scale to wild and domestic birds worldwide. Compared to the parent clade 2.3.4.4A viruses (designated H5N8-A) in 2013/2015, viruses in clade 2.3.4.4B (designated H5N8-B) from 2015 to 2021 caused high mortality in wild birds and Pekin ducks and was isolated recently from humans and dead seals. Therefore, there is an urgent need to keep vigilance to assess periodically the virulence and transmissibility of H5N8 in different animals and understand the molecular mechanisms underlying potential virus adaptation. In this comprehensive study, we determined the pathogenicity and virulence markers of H5N8-A and H5N8-B in chickens, ducks and mice and determined the genetic markers for the efficient transmission of H5N8 viruses in Pekin ducks and chickens.

METHODS
Recombinant H5N8-A, H5N8-B and H5N8-A viruses carrying gene segment(s) from H5N8-B were successfully constructed using reverse genetics. Replication efficiency in avian and mammalian cells, receptor-binding affinity, sialidase and polymerase activities, interferon (IFN)-antagonism and apoptosis induction were assessed in vitro. In vivo, chickens and ducks (n=10 each) were inoculated oculonasal with recombinant viruses and one-day post-inoculation five birds were added to assess chicken-to-chicken or duck-to-duck transmission, respectively. Morbidity, mortality, virus excretion, tissue distribution and histopathological alterations were described. Furthermore, in mice, we assessed the impact of selected recombinant H5N8-B viruses on bodyweight gain, mortality, virus replication in the lungs and interferon induction.

RESULTS
In vitro, compared to H5N8-A, H5N8-B virus exhibited increased binding affinity to avian-sialic acid receptors and higher NA activity. In duck cells, but not in chicken or human cells, H5N8-B replicated at higher levels than H5N8-A. Both H5N8-A and H5N8-B blocked IFN-β induction efficiently despite of the striking preferential differences in the NS1 C-terminus domain (CTE). In chickens, both viruses were highly virulent, while H5N8-B was more virulent in Pekin ducks and mice than H5N8-A. In ducks, HA, NA and NS played a role in H5N8-B virulence and transmission. Mutations in the NS1 CTE of H5N8-B (i) reduced virus shedding, transmission and/or endotheliotropism in chickens and ducks, (ii) reduced virulence and replication in mice and (iii) contributed to the high efficiency of H5N8-B for blocking IFN and apoptosis inductions in human lung cells indicating a role in virus adaptation in mammals.

CONCLUSION
The recent H5N8-B virus exhibited high virulence in chickens. Pekin ducks and mice compared to the predecessor H5N8-A virus. NS segment contributes to H5N8-B fitness in birds and mammals.
A history of obesity reduces the immune response to influenza virus in a non-canonical NLRP3-dependent manner

**Topic:** Viral and host factors in the pathogenesis of influenza, RSV disease and COVID-19

**Katina Hulme** (1), Ellesandra Noye (1), Conor Bloxham (2), Nathalie Verzele (1), Marcus Tong (1), Larisa Labzin (3), Keng Yi Chew (1), Helle Bielefeldt-Ohmann (1,4), Kate Schroder (3), Kirsty Short (1,4)

1: School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, Australia; 2: School of Biomedical Science, University of Queensland, Brisbane, Australia; 3: Institute of Molecular Biosciences, University of Queensland, Brisbane, Australia; 4: Australian Infectious Diseases Research Centre, The University of Queensland, Brisbane, Qld, Australia

**BACKGROUND**

Obesity significantly increases the risk of death following an influenza virus infection. Consistent with these clinical observations, we and others have shown that mice with diet-induced obesity develop much more severe influenza than their lean-fed counterparts. Traditionally, it has been assumed that this increased susceptibility can be reversed by weight loss. However, this remains to be tested experimentally.

**METHODS**

Here, a novel mouse model was developed to study the long-term effects of obesity on anti-viral immunity. Four-weeks old C57BL/6 mice were fed a high fat or lean diet for 10 weeks. After 10 weeks, mice fed a high-fat diet had a significantly higher total body weight and percentage body fat compared to mice fed the lean diet. Obese mice were then swapped to a lean diet for 10 weeks.

**RESULTS**

After 10 weeks on the lean diet, mice that were previously obese (PO) had an equivalent body weight and percentage body fat to mice that received the lean diet for the entirety of the 20-week treatment period. However, upon infection with influenza virus (A/Auckland/09[H1N1]), PO mice displayed increased viral replication, lung inflammation, body weight loss and pulmonary dysfunction compared to lean-fed mice. Alveolar cells of PO mice also had an altered metabolic state compared to those of lean fed mice. Importantly, deficiency in either NLRP3 or caspase-11 blocked the long-term effect of murine obesity on susceptibility to severe influenza virus infection.

**CONCLUSIONS**

We propose that obesity can have long-term, non-canonical NLRP3 dependent, effects on the metabolism of innate inflammatory cells rendering their anti-viral responses impaired. Understanding the long-term effects that obesity has on anti-viral immunity will help pave the way for development of novel therapeutics to improve the health of the billions of people who are, or previously have been, obese.
Silencing pulmonary sensory neurons impact influenza pathogenesis

**Topic:** Viral and host factors in the pathogenesis of influenza. RSV disease and COVID-19

**Nathalie A. J. Verzele** (1, 2), Matthew W. Trewella (1), Eloise M. Whitehead (1), Stuart B. Mazzone (1), Kirsty R. Short (2), Alice E. McGovern (1)

1: Department of Anatomy and Physiology, The University of Melbourne, Australia; 2: School of Chemistry and Molecular Biosciences, The University of Queensland, Australia

**BACKGROUND**

During influenza A virus (IAV) infection, the driver of disease severity is often an aberrant inflammatory response with numerous different cell types contributing to the pathogenesis. The lungs are densely innervated by pulmonary sensory neurons with nerve terminals extending into the alveolar airspace and the cell body located at the base of the skull in the vagal sensory ganglia. The nerve terminals express multiple receptors that sensitize the nerves, making them critical for detecting changes within the respiratory tract. However, to date, the contribution of these to IAV disease severity is yet to be investigated. We recently showed that during IAV respiratory viral infection these pulmonary sensory neurons undergo significant changes and take on a neuroinflammatory phenotype, particularly in response to the aberrant inflammation within the lungs. Here, we sought to provide the first insight as the role pulmonary sensory neurons play in IAV pathogenesis and disease severity.

**METHODS**

Using a murine model (C57Bl/6 mice, 8-10 weeks age) of IAV respiratory infection (Auckland/1/09 H1N1, 6×10^5 PFU), pulmonary sensory neuron activity (specifically, TRPV1+ neurons expressing the NaV1.8 channel) was silenced by twice daily inhaled treatment with the drug QX-314 (100µM) from 3 days post-infection. Disease severity was measured using whole body plethysmography, clinical scoring, gene expression and cytokine levels.

**RESULTS**

Silencing the activity of pulmonary sensory neurons with QX-314 in IAV-infected mice resulted in a more severe weight loss and increased clinical symptoms compared to vehicle treated IAV-infected mice. Additionally, QX-314 treatment in IAV-infected mice resulted in increased pulmonary concentrations of IL-6 compared to vehicle. However, no significant differences in breathing parameters such as respiratory rate and tidal volume were observed between treatments in IAV-infected mice. The vagal sensory ganglia, which contain the pulmonary sensory neurons showed a significant increase in the expression of interferon stimulated genes Ifi19 and Ifi20 in QX-314 treated IAV infected mice compared to vehicle. In addition, the neuropeptide genes Calca and Tac1 were significantly decreased indicating a potential change in inflammatory modulating neuropeptide release in the pulmonary system. Finally, the ganglia showed a significant increase in P2x3 expression, a key receptor via which the epithelial ATP activates vagal sensory neurons.

**CONCLUSION**

Together, these data raise the possibility that the vagal sensory ganglia and pulmonary sensory neurons play an active role in regulating IAV pathogenesis. Modulation of their activity may therefore be a promising novel therapeutic approach to reduce the severity of influenza virus infection.
Mapping influenza A virus cellular defense mechanisms reveals a strategy for evasion of autophagy

**Topic:** Viral and host factors in the pathogenesis of influenza, RSV disease and COVID-19

Laura Martin-Sancho (1), Shashank Tripathi (2), Ariel Rodriguez-Frandsen (1), Maite Sanchez-Aparicio (3), Michael McGregor (4), Kelsey Haas (4), Danielle Swaney (4), Thong Nguyen (4), João Mamede (5), Christopher Churas (6), Dexter Pratt (6), Sara Rasenthal (6), Laura Riva (1), Courtney Nguyen (1), Nish Beltran-Raygoza (1), Stephen Seanthanavichiar (1), Guojun Wang (3), David Jimenez-Morasles (4), Paul De Jesus (1), Hong Moulton (7), David Stein (7), Lars Pache (1), Max Chang (6), Chris Benner (6), Trey Ideker (6), Randy Albrecht (3), Judd Hultquist (8), Evan Krogan (4), Adolfo García-Sastre (3), Sumit Chanda (1)

1: Scripps, United States of America; 2: Indian Institute of Science, India; 3: Icahn School of Medicine at Mount Sinai, United States of America; 4: University of California San Francisco, United States of America; 5: Rush University Medical Center, United States of America; 6: University of California San Diego, United States of America; 7: Oregon State University, United States of America; 8: Northwestern University Feinberg School of Medicine, United States of America

**BACKGROUND**

In response to viral infection, the host cell deploys a diverse set of mechanisms designed for pathogen clearance. Thus, survival of viruses in the host cell depends on their ability to evade these cellular defenses. Understanding of these complex interactions can provide critical information on viral replication and pathogenic mechanisms and assist with development of antiviral strategies.

**METHODS**

To identify virus-host interactions that control influenza A virus (IAV) replication, we conducted genome-wide functional genetic screens in human macrophage-like cells. These data were combined with transcriptomics of IAV-infected cells, proteomics, and mechanistic studies, to reveal potential viral evasion strategies.

**RESULTS**

Integration of the multiOMICs data revealed the diverse cellular landscape of interferon-dependent and -independent mechanisms that act to inhibit IAV replication. These included autophagy, an important cellular defense strategy for which we lack molecular understanding of viral antagonism. One of the factors identified in the screen to control IAV replication was the autophagy regulator TBC1D5-Related Domain Family Member 5 (TBC1D5). These results were reproduced using in vitro, ex vivo, and in vivo systems, further highlighting the importance of this host factor. To control cellular homeostasis or degradation of invading pathogens, autophagosomes engulf cellular or viral cargo and fuse with lysosomes to degrade its content. This autophagosome-lysosome fusion event depends on the interaction between TBC1D5 and the small GTPase Rab7. Notably, we found that in IAV-infected cells the interaction of TBC1D5 and Rab7 was abrogated. This event was mapped to IAV M protein, as a wild-type but not an M-deicient IAV reduced TBC1D5-Rab7 association and prevented fusion of autophagosomes with lysosomes, enabling M2 to traffic to the cell membrane to support IAV budding and growth.

**CONCLUSIONS**

Taken together, this study identifies M2 interference with TBC1D5-Rab7 interaction as a novel viral evasion strategy to misregulate autophagy and subvert degradation. Two bacteria have been also shown to target Rab7 and/or TBC1D5 to promote survival, suggesting this is a convergent evolutionary mechanism exploited by both bacteria and viruses to evade autophagy. Therapeutic targeting of this critical node may be an important strategy for the development of broad-spectrum antivirals that act to restore lysosomal degradation of viral cargo.
Influenza NS1 protein epigenetically upregulates microRNA-146a to suppress inflammatory responses and promote viral infection

**Topic:** Viral and host factors in the pathogenesis of influenza, RSV disease and COVID-19

Bobo Wing Yee Mok, Conor J Cremin, Honglian Liu, Honglin Chen
State Key Laboratory for Emerging Infectious Diseases and Department of Microbiology, Li Ka Shing Faculty of Medicine, the University of Hong Kong, Hong Kong SAR

**BACKGROUND**
Increasing evidence suggests that host microRNAs (miRNAs) play different roles in viral life-cycles. Expression of miRNAs can be involved in antiviral responses, and/or promoting infection through complex regulatory pathways. miR-146a-5p, a host miRNA that is induced primarily by NF-kB, and other host transcription factors, was described as a pro-viral factor of viral infection for many viruses, including influenza viruses.

**METHODS AND RESULTS**
Transcriptome analysis of influenza virus-infected lung epithelial cells identified that miR-146a-5p is one of the most upregulated miRNAs upon infection, and most significantly, such induction was observed only in the presence of the viral non-structural protein 1 (NS1) in the nucleus of infected cells. We found that miR-146a-5p promotes influenza virus via modulation of the STAT1 signaling pathway: depletion of miR-146a-5p by inhibitors or CRISPR-knockout attenuated viral replication in cells while introducing miR-146a-5p mimic in mouse respiratory organs alleviated pathogenic outcomes of the animals infected with highly pathogenic influenza viruses. Mechanistically, we found that F103/M106 residues of the NS1 protein are important for miR-146a-5p induction, by deregulating the expression and function of cellular CSFP, which negatively affect miR-146a-5p expression. Further analysis revealed that the enhancement of miR-146a-5p expression by NS1 is contributed through its interaction with an epigenetic regulator, Bromodomain protein 4 (BRD4). Surprisingly, treatment of JQ1, a BRD4 inhibitor, can elevate the expression of small form BRD4, which works synergistically with NS1 to enhance miRNA-146a-5p expression.

**CONCLUSION**
Our data reveal an unrecognized function of NS1 to epigenetically upregulate specific host miRNAs for regulating host immune response and thereby facilitate viral replication.
Precise monitoring of COVID-19 severity and treatment

Topic: Viral and host factors in the pathogenesis of influenza, RSV disease and COVID-19

Andre F. Rendeire (1,2), Charles Kyrakos Vorkas (3), Jan Krumsieck (1,2), Harjot Singh (3), Shashi N. Kapalia (3), Luca Vincenzo Cappelli (4), Maria Teresa Cacciaferril (5), Giorgio Inghirami (4), Olivia Elemento (1,2), Mirella Salvatore (3,5)

1: Institute for Computational Biomedicine, Department of Physiology and Biophysics, Weill Cornell Medicine, New York, NY, USA;
2: Caryl and Israel Englander Institute for Precision Medicine, Weill Cornell Medicine, New York, NY, USA;
3: Department of Medicine, Weill Cornell Medicine, New York, NY, United States of America;
4: Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, NY, USA;
5: Department of Population Health Sciences, Weill Cornell Medicine, New York, United States of America

BACKGROUND

The pandemic caused by infection with the severe acute respiratory coronavirus type 2 (SARS-CoV-2) has infected more than 218 million people worldwide as of August 2021, caused more than 4.5 million deaths, and strains health systems on an unprecedented scale. Several common laboratory tests have been used to monitor COVID-19, with their levels varying associated with disease severity. However, while routinely available assays depict an incomplete landscape of pathophysiological changes associated with COVID-19, one possible approach is to increase the dimensionality of the system using mixed-modality profiling. While much work has been done on the characterization of the host immune response through cytometric or serological methods, characterization of the metabolic state of COVID-19 patients has just begun.

METHODS

We conducted an observational study of 75 individuals with acute or convalescent COVID-19 that were treated as in- or out-patients between April and July 2020. The disease was categorized using World Health Organization disease severity scale for the prognostication of COVID-19 patients. Serum samples were collected at hospital admission, when permissible approximately every 7 days thereafter, and for convalescent patients as outpatients at least 90 days from symptom onset (129 samples from 75 patients, 32 convalescent). We performed targeted high-throughput NMR-based detection of metabolites in circulating blood serum (Nightingale Health Ltd.) (Figure 1A). The NMR assay detected 168 metabolite species in absolute molar quantities, and 81 additional measurements of relative proportions covering diverse metabolic species such as lipids and fatty acids, apolipoproteins, amino acids, ketone bodies, and other molecules with known prognostic value across various diseases.

RESULTS

To identify the metabolic features associated with COVID-19 outcome, we leveraged linear mixed effect models to explain COVID-19 disease severity as a function of metabolite levels independently from patient age, gender, race, and body mass index (BMI) (Figure 1b). While most of the 249 metabolite species known prognostic value across various diseases such as albumin, creatinine, and apolipoproteins showed no association with disease severity as measured by the WHo score, we found significant associations for 56 metabolites (p < 0.05, adjusted for multiple testing with the Benjamini Hochberg False Discovery Rate method), which were dominated by lipid and lipoprotein subclasses. We found that Albumin, high-density lipoprotein (HDL) and small HDL, particle species, as well as the cholesteryl-ester component of HDL and intermediate-density lipoproteins (IDL) declined proportionally with the increase in WHO score with steeper decline in the most severe cases (Figure 1c). On the other hand, extra small, very low-density lipoprotein (VLDL), particles with increased phospholipids component and extra-small VLDL, IDL, LDL and HDL with increased triglycerides were correlated with increased severity. The acetylated glycoproteins (GlycA), phenylalanine, and fraction of monounsaturated fatty acids (MUFA) acetoacetate, and the ratio of apolipoprotein B to A1 to disease severity.

We also assessed metabolic changes associated with treatment with tocilizumab, an inhibitor of the proinflammatory interleukin-6 was used in COVID-19 patients with elevated inflammatory markers and rapidly escalating oxygen requirements. We fit a linear model on the time since treatment with age, gender, race, BMI, and disease severity as covariates. Several metabolite species significantly associated with tocilizumab treatment (Figure 1e). Some metabolite species that were significantly changed with COVID-19 severity were changed by tocilizumab treatment. However, we also observed metabolite species that were significantly changed with COVID-19 severity with no apparent change with tocilizumab treatment (Figure 1e).

DISCUSSION

Here we present longitudinal immuno-metabolic data on a cohort of COVID-19 patients of different disease severity. We show that patient metabolism during disease is quite dynamic, reflecting disease progression and treatment. We identified a deep alteration of the lipoprotein particles levels and composition. Furthermore, we developed tools to precisely monitor patient trajectories using metabolic data, enabling risk assessment on a continual fashion, and a novel patient stratification strategy. It is plausible that cytokine modulation of key metabolic enzymes or energy usage by the immune system during acute infection are a major source of the metabolic changes associated with COVID-19. Additional evidence of immune influence on metabolism in our data is the fact that tocilizumab-a neutralizing antibody of the pro-inflammatory IL-6 partially rescues the effect of disease severity at the metabolic level reinforcing the idea that metabolic changes during COVID-19 are likely to be at least partially driven by the immune system.
Safety, tolerability and pharmacokinetics of ensovibep in patients with mild to moderate COVID-19 - preliminary report of a phase 2a, open-label, single dose escalation study.

**Topic:** Antiviral and immune therapy for influenza, RSV disease and COVID-19


1: Centre for Human Drug Research (CHDR), Leiden, The Netherlands;
2: Department of Infectious Diseases, Leiden University Medical Center (LUMC), Leiden, The Netherlands;
3: Molecular Partners AG, Schlieren, Switzerland

**BACKGROUND**

There is an urgent need for treatment of mild-to-moderate COVID-19 to prevent disease progression. Ensovibep is a multi-domain DARPin® molecule that has three different paratopes that bind to the receptor binding domain (RBD) of the SARS-CoV-2 spike protein, and contains two, half-life extending, human serum albumin binding DARPin® domains. Ensovibep has several potential benefits, including high affinity and strong binding to the SARS-CoV-2 S-protein, and low likelihood of escape RBD mutations due to its unique structure. We present preliminary observations of the safety and tolerability (to Day 91), pharmacokinetics and viral dynamics (to Day 15) of two doses of ensovibep in mild-to-moderate symptomatic COVID-19 patients.

**METHODS**

This was an open-label, non-comparative, intravenous single-dose escalation study performed at the Leiden University Medical Center. Patients were referred by the municipal healthcare service and were eligible if they had ≥1 mild-to-moderate COVID-19 symptoms and positive rapid antigen test prior to dosing. Two doses of ensovibep ([225 mg](n=6), 600 mg[n=6]) were evaluated. Safety and tolerability were assessed by vital signs, physical examination, blood chemistry/haematology and Visual Infusion Phlebitis (VIP) scale. Pharmacokinetics and viral load (nasopharyngeal qPCR) were assessed pre-dose and at selected time points (Figure 1).

**RESULTS**

Baseline characteristics are listed in Table 1. There were neither safety findings of clinical concern nor injection site reactions observed up to Day 91. Adverse events were of mild (11/16) to moderate (5/16) severity. Headache was the most (4/16). Four and three subjects reported ≥1 AE in the 225 mg and 600 mg group, respectively. Pharmacokinetic analysis showed maintained drug exposure in all patients through Day 15 and comparable kinetic profiles within dose groups. Viral load showed a consistent decline. By Day 15, viral load decreased in all patients and was below the LLOQ in 2/6 and 5/6 patients of the 225 mg and 600 mg group, respectively.

**CONCLUSION**

Single ensovibep doses at 225 mg and 600 mg were safe and well tolerated, showed favourable pharmacokinetics and encouraging viral load reduction was observed. These findings support large-scale controlled evaluation of ensovibep as a treatment for mild-to-moderate COVID-19.

**Table 1. Baseline characteristics**

<table>
<thead>
<tr>
<th>Sex, n (female) (%)</th>
<th>Cohort 1, 225mg (n=6)</th>
<th>Cohort 2, 600mg (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23 (21-25)</td>
<td>24 (23-28)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26 (24-28)</td>
<td>25 (23-27)</td>
</tr>
<tr>
<td>Days between symptom onset and dosing</td>
<td>5 (4-6)</td>
<td>5 (4-5)</td>
</tr>
<tr>
<td>Median qPCR result (copies/mL)</td>
<td>4 (325)</td>
<td>4 (1025)</td>
</tr>
</tbody>
</table>

Data are presented as median (interquartile range) unless indicated otherwise. BMI, body mass index; qPCR, quantitative polymerase chain reaction.

**Figure 1.** Data represent median ensovibep serum concentration (red: 600 mg; blue: 225 mg). Horizontal lines in each box plot represent median viral load and interquartile range. Whiskers represent minimum and maximum value (red 600 mg; blue: 225 mg). Dotted black line denotes lower limit of quantification of the assay measuring viral load (LLOQ, 42 ≥ 0.8 log10 copies/mL). Samples reported as <LLOQ were replaced by LLOQ/2 (1.04 log10 copies/mL).
Host- and viral-targeted antiviral development for SARS-CoV-2 and influenza virus, treating the current pandemic and preparing for the next

**Topic:** Antiviral and immune therapy for influenza, RSV disease and COVID-19

Kris White (1), Romel Rosales (1), Briana McGovern (1), Khushboo Bafna (2), Michael Williams (1), Robert Krug (3), Kevan Shokat (4), Brian Shoichet (4), Gaetana Montelione (2), Nevan Krogan (4), Adolfo Garcia Sastre (1)

1: Kahn School of Medicine at Mount Sinai, United States of America; 2: Department of Chemistry and Chemical Biology, Center for Biotechnology and Interdisciplinary Sciences, Rensselaer Polytechnic Institute, Troy, New York, 12180; 3: Department of Molecular Biosciences, John Ring LaMontagne Center for Infectious Disease, Institute for Cellular and Molecular Biology, University of Texas at Austin, Austin, Texas 78712 USA; 4: Quantitative Biosciences Institute (QBI), University of California San Francisco, San Francisco, CA, USA.

**BACKGROUND**
The ongoing COVID-19 pandemic is the result of a zoonotic transmission event, similar to previous coronavirus epidemics. Antiviral therapeutics are urgently needed to combat SARS-CoV-2 in the current pandemic and will be the first line of defense for the future coronavirus and influenza virus pandemics. The SARS-CoV-2 coronavirus is reliant on host cell proteins to successfully complete the viral replication cycle. Our previously published SARS-CoV-2 interactome highlighted 332 host proteins that are likely to play a role in the viral life cycle of SARS-CoV-2. We have continued to use the ever-expanding knowledge of the SARS-CoV-2 life cycle in search of current clinically-approved drugs that can be repurposed as host-targeted or viral-targeted antivirals for the treatment of COVID-19. The goal of our lab is to produce SARS-CoV-2 and influenza virus antiviral therapeutics that take advantage of synergistic interactions and most importantly, prevent the rise of resistant variants.

**METHODS**
We tested over 1000 existing drugs that target identified host and viral proteins, with many of these drugs showing significant antiviral activity against SARS-CoV-2 in cell culture. We have begun to dissect the potential mechanism of action from our most potent hits, which we hope will lead to insights into SARS-CoV-2 biology and the identification of further drugs with repurposing potential. We have also explored drug combination studies, with a focus on finding synergistic interactions with remdesivir. We are now focused on using animal models of SARS-CoV-2 infection to determine the in vivo efficacy of our top clinically-approved repurposing candidates in the support of ongoing clinical trials.

**RESULTS**
Thus far we have screened over 1000 drugs against SARS-CoV-2 in cell culture. In this manner we have identified over 60 compounds which inhibit SARS-CoV-2 with a sub-micromolar IC₅₀ in vitro, with our top host-targeted hit having a sub-nanomolar IC₅₀. We have also found that multiple FDA-approved HCV protease inhibitors have antiviral activity against SARS-CoV-2 and synergize with the current standard of care, remdesivir, through targeting the SARS-CoV-2 PLpro enzyme. Finally, we have tested our most potent clinically-approved host-targeted antivirals in animal models of SARS-CoV-2 infection with reductions in viral lung titers and SARS-CoV-2 associated histopathology.

**CONCLUSIONS**
Our results offer multiple clinically-approved drugs with significant potential to impact patient health in the ongoing COVID-19 pandemic. The host-targeted antivirals we have identified have the potential to be an off-the-shelf option for future coronavirus and influenza virus pandemics, with broad-spectrum activity. Furthermore, the synergistic interaction we have identified between SARS-CoV-2 PLpro inhibitors and viral polymerase inhibitors should be of great value toward the development of SARS-CoV-2 specific antiviral cocktails. We hope that our work will offer many insights into SARS-CoV-2 biology that can be used in the specific design of future antivirals for the treatment of coronavirus infection.
LAT8881 and other naturally derived cytokine peptides limit respiratory virus replication and severe disease

**Topic:** Antiviral and immune therapy for influenza, RSV disease and COVID-19

Christopher Harpur (1,2), Mélanie Le Page (1,2), Andrew Gearing (3), Michelle Tate (1,2)

1: Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, Victoria, Australia;
2: Department of Molecular and Translational Sciences, Monash University, Clayton, Victoria, Australia;
3: Lateral Pharma Pty Ltd, Melbourne, Victoria, Australia

**BACKGROUND**

There is an urgent need to develop new therapeutics that safely limit the severity of respiratory viral infections including IAV, RSV and SARS-CoV2. Severe and fatal infections are associated with significant viral replication and damaging hyperinflammatory responses which can lead to morbidity, mortality and long-term multi-system organ damage. LAT8881 is a 16 amino acid synthetic form of the naturally occurring C-terminal fragment of growth hormone (GH). LAT8881 has been previously shown to act independently of the GH receptor to reduce inflammatory damage and promote tissue repair in a rabbit model of arthritis. Additionally, LAT8881 has been investigated in several clinical trials in healthy volunteers and for the treatment of obesity and has an established safety record. In light of its effects in improving inflammatory damage in animal models, we investigated the potential of LAT8881 and related compounds as a treatment for severe respiratory viral infections.

**METHODS**

We utilised a pre-clinical mouse model of severe IAV infection (10^6 PFU HKx31 H3N2) to evaluate the therapeutic potential of intranasal delivery of the synthetic GH fragment LAT8881 (10 or 20 mg/kg). Additionally, LAT9991F, the truncated form and known stable metabolite of LAT8881 was also tested. Disease severity was evaluated by examining weight loss, survival, viral loads, as well as inflammation in the airways and sera. RNA was extracted from lung tissues and comprehensive transcriptome analysis performed by RNAseq.

**RESULTS**

Daily intranasal treatment of mice with LAT8881 from 1 day following IAV infection significantly increased the survival of mice, which was associated with reduced infectious viral loads, cellular infiltrates, vascular permeability, as well as cytokines in the airways and serum. Interestingly, LAT8881 and LAT9991F had no impact on IAV in vitro replication in primary human bronchial epithelial cells, suggesting the compounds do not elicit direct anti-viral responses. RNAseq analysis of mouse lung tissues suggested that LAT8881 broadly modulates host defence responses and initial studies in mouse models of RSV infection have confirmed the broad applicability of LAT8881 and other related peptides. Current studies are utilising a photo-activatable version of active peptide to pull down and identify potential binding partners in lung tissues and airway epithelial cell lines.

**CONCLUSIONS**

LAT8881 (and its metabolite LAT9991F) are potential new therapeutics that limit viral replication and protect the lung from hyperinflammation and severe disease. Importantly, as synthetic peptides with an established clinical safety profile in humans, these data warrant further clinical studies investigating their potential as therapeutics for respiratory virus infections.
LAT8881, a novel human growth hormone fragment, is highly effective in reducing SARS-CoV-2 inflammation and lethality in a K18-hACE2 mouse model

**Topic:** Antiviral and immune therapy for influenza, RSV disease and COVID-19

**Matt D. Johansen (1), Stefan Miemczyk (1), Duc H. Nguyen (1), Nicole G. Hansbro (1), Andrew Gearing (2), Bernadette M. Saunders (1), Warwick J. Britton (1), Philip M. Hansbro (1)**

1: University of Technology Sydney/Centenary Institute, Australia; 2: Lateral Pharma, Melbourne, Australia

**BACKGROUND**
Since the emergence of SARS-CoV-2 in late 2019, there have been 235 million cases and 4.8 million deaths reported globally due to COVID-19 (Sep 2021). Disease progression is largely driven by virally-induced inflammation triggered by a cytokine storm, creating a highly pro-inflammatory environment that can lead to acute respiratory distress syndrome (ARDS), multi-organ failure and death. Despite intensive global vaccination regimes, there is a significant gap in the availability of new and emerging therapeutics, which can be implemented to reduce inflammation and mitigate disease severity in COVID-19 patients. The objective of this study was to evaluate the therapeutic role of LAT8881, one of a novel class of human growth hormone fragments in reducing inflammation in a COVID-19 mouse model.

**METHODS**
Female hemizygous K18-hACE2 mice (Tg(K18-ACE2):Prlmn) were intranasally inoculated with 10^3 PFU of SARS-CoV-2 Wuhan isolate (VIC2020). At 1-day post-infection (dpi), mice were treated with 20 mg/kg of LAT8881, a 16 amino acid C-terminal fragment of human growth hormone, via intranasal or intraperitoneal administration every second day. At day 6 post-infection, mice were sacrificed and bronchoalveolar lavage fluid (BALF) was collected from the single lobe lungs, while multi-lobe lungs were used to determine lung viral titres using plaque assay. The single lobe lungs were also used for histological analysis.

**RESULTS**
Intranasal delivery had no effect on weight loss, clinical presentation, lung inflammation or viral titres in the lungs during the peak of inflammation at 6 dpi. However, we found that intraperitoneal administration of LAT8881 (duplicate experiments) significantly reduced inflammatory cell counts in the BALF and significantly improved clinical presentation of infected mice. Furthermore, we identified significantly decreased viral titres in the BALF of mice treated with LAT8881 via intraperitoneal administration. Importantly, we found that 50% of mice that received intraperitoneal administration of LAT8881 following SARS-CoV-2 infection survived the infection and cleared the virus over a 14 day period. Further analyses determined that there was a marked reduction in the cytokine profile of mice treated with LAT8881 via intraperitoneal administration compared to SARS-CoV-2 sham-treated groups.

**CONCLUSIONS**
We conclude that LAT8881 is a promising candidate for the treatment of SARS-CoV-2-induced inflammation and warrants further investigation as a potential treatment for COVID-19.
Local delivery of interferon beta (SNG001) by inhalation upregulates lung antiviral biomarkers

**Topic:** Antiviral and immune therapy for influenza, RSV disease and COVID-19

David Singh (1), Tom Wilkinson (2,3), Iona Beegan (4), Kerry Lunn (5), Sophie Reynolds (6), Pedro Rodrigues (7), Sandy Atkin (8), Sarah Dudley (9), Victoria Tear (10), Phillip David Monk (11), Jody Brookes (12), Marcin Markowski (13), Donna Davies (14), Stephen Holgate (15), Ratko Djukanovic (16)

1: Medicines Evaluation Unit, Manchester University NHS Foundation Trust & University of Manchester, Manchester, UK; 2: Clinical & Experimental Sciences, University of Southampton Faculty of Medicine, Sir Henry Wellcome Laboratories, Southampton General Hospital, Tremena Road, Southampton, SO16 6YD, UK; 3: Southampton NHS Respiratory Biomedical Research Unit, Southampton General Hospital, Southampton, UK; 4: Synairgen Research, Southampton General Hospital, Southampton, UK; 5: Synairgen Research, Southampton General Hospital, Southampton, UK; 6: Synairgen Research, Southampton General Hospital, Southampton, UK; 7: Synairgen Research, Southampton General Hospital, Southampton, UK; 8: Synairgen Research, Southampton General Hospital, Southampton, UK; 9: Synairgen Research, Southampton General Hospital, Southampton, UK; 10: Synairgen Research, Southampton General Hospital, Southampton, UK; 11: Synairgen Research, Southampton General Hospital, Southampton, United Kingdom; 12: Synairgen Research, Southampton General Hospital, Southampton, UK; 13: TranSeal, Wokingham, UK; 14: NHRR Biomedical Research Centre, University Hospital Southampton, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Sir Henry Wellcome Laboratories, Southampton, UK; 15: Synairgen Research, Southampton General Hospital, Southampton, UK; NHRR Southampton Biomedical Research Centre, University Hospital Southampton, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Sir Henry Wellcome Laboratories, Southampton, UK; 16: NHRR Southampton Biomedical Research Centre, University Hospital Southampton, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Sir Henry Wellcome Laboratories, Southampton, UK.

**BACKGROUND**

New therapies for severe viral lung infections are needed. Interferon beta (IFN-β) is a naturally occurring protein that is an essential driver of host antiviral responses and has antiviral activity against SARS-CoV-2. The SARS-CoV-2 virus suppresses IFN-β production by cells to evade the host's immune response. SNG001 is an inhaled formulation of recombinant IFN-β (IFN-β-1a), a lung-targeted, host-directed, broad-spectrum, SARS-CoV-2 variant-agnostic, antiviral drug in development for COVID-19. The aim of treatment with inhaled SNG001 is to achieve a high concentration of IFN-β at the site of infection in the lungs to drive antiviral pathways. To demonstrate that local delivery can drive robust and clinically relevant antiviral responses in the lungs, the levels of IFN- dependent antiviral biomarkers have been assessed in sputum samples collected from patients after receiving SNG001.

**METHODS**

Sputum samples were collected prior to and 24 hours after dosing with SNG001 in two phase 2, double-blind, placebo-controlled RCT in asthma and COPD patients (SG005, NCT01261177; SG015, NCT03570359). Sputum cell gene expression of IFN-β dependent antiviral biomarkers, including MX1 and OAS1, was determined by RT-qPCR.

**RESULTS**

Inhaled SNG001 was well tolerated and significantly upregulated lung antiviral biomarker responses. Biomarker responses were similar in younger asthmatics and older COPD patients (Figure 1).

**CONCLUSIONS**

IFN-β delivered directly to the lungs, in the form of SNG001, was well tolerated and boosted lung antiviral activity irrespective of age, offering a potential therapeutic option in hospitalized COVID-19 patients. The ability of SNG001 to stimulate antiviral responses in the lungs and encouraging data from a placebo-controlled RCT of SNG001 in hospitalized COVID-19 patients stand in contradiction to a trial of subcutaneously delivered IFN-β which failed to show clinical benefit in COVID-19. The difference is likely related to the much higher local concentrations of IFN-β in the lungs achieved via inhalation compared to a systemic route of administration.

**References**


*Figure 1: Upregulation of IFN-β dependent antiviral biomarkers in sputum cells following inhaled administration of SNG001*
Safety, tolerability and biological activity of repeated intranasal administration of Ampligen (Poly I:Poly C12U) as potential antiviral treatment in healthy subjects

**Topic:** Human influenza, RSV disease and COVID-19 challenge studies

Johan L. van der Plas (1,2), Lisanne C.A. Smit (1,2), Aliede E. in't Veld (1), Christina Ylanti (1), Ingrid M.C. Kamering (1,2), Naomi B. Klarenbeek (1,3), Diane L. Young (4), David R. Strayer (4), Manon A.A. Jansen (2), Matthijs Moerland (1)

1: Centre for Human Drug Research, Leiden, The Netherlands; 2: Department of Infectious Diseases, Leiden University Medical Centre, Leiden, The Netherlands; 3: Department of Internal Medicine, Leiden University Medical Centre, Leiden, The Netherlands; 4: AIM ImmunoTech Inc., Ocala, Florida, United States

**BACKGROUND**

Rintatolimod (Ampligen®) is a synthetic double-stranded RNA (Poly I: Poly C12U) known to act as a selective Toll-like receptor 3 (TLR3) agonist that drives a type I interferon response. Antiviral activity of rintatolimod against coronaviruses (including SARS-CoV-2) has been shown in preclinical studies. Intranasal administration of rintatolimod could induce a protective innate immune response and thereby inhibit viruses at the point of entry. Therefore, rintatolimod has the potential to be developed as prophylactic or early treatment against COVID-19 and other respiratory viral infections. This study aimed to assess the safety, tolerability and biological activity of a 13-day dosing regimen for intranasal rintatolimod.

**METHODS**

This was a randomized, double-blind, placebo-controlled, dose escalation study performed at the Centre for Human Drug Research, the Netherlands. Rintatolimod (up to doses of 1250 µg) or placebo (4:1) was administered in both nostrils every other day (q.o.d.) for a total of 7 doses. Safety and tolerability were assessed by vital signs, physical examination (including anterior rhinoscopy), chemistry and hematology blood tests, adverse events (AEs) and self-reported local symptoms (mild-moderate-severe) and pain (rating scale, 0-10). To characterize the innate mucosal immune response, mucosal samples were obtained on selected time points (Figure 1). The mucosal lining fluid will be analyzed using a cytokine panel that includes type I interferons (IFN-α, IFN-β) and NFκB-mediated cytokines (IFN-γ, IL-2, IL-8, TNFα) and mucosal immune cells (granulocytes, T-cells, B-cells, dendritic cells, macrophages/monocytes) will be characterized using spectral flow cytometry.

**RESULTS**

In total 40 male and female subjects, aged 18 - 69 years were included (Figure 2). Repeated intranasal administration of rintatolimod was well tolerated with no findings of clinical concern. Thirty-eight post-dose AEs were reported by 16 (50%) exposed subjects and were of mild-to-moderate severity. No dose related trends or dose limiting toxicities were identified. Analysis of mucosal innate immune responses is currently ongoing.

**CONCLUSIONS**

Repeated intranasal administration of rintatolimod q.o.d was well tolerated (up to doses of 1250µg). Pending results on mucosal biological activity of rintatolimod may help to optimize dose decision for phase II development as prophylaxis or early treatment against SARS-CoV-2 and other respiratory viruses.

![Figure 1 - Schedule of assessment for characterization of biological activity of intranasal rintatolimod](image-url)
Figure 2 – Consort flow diagram
Robust neutralizing antibody and cell mediated immune responses raised by sa-mRNA influenza vaccines co-expressing HA and NA antigens

**Topic**: Human influenza, RSV disease and COVID-19 challenge studies

Giuseppe Palladino, Gillis Otten, Ethan Settembre, Yingxia Wen

Serafim, United States of America

**BACKGROUND**

Vaccination is the most cost-effective strategy to combat the substantial morbidity and mortality caused by influenza infection which is responsible for seasonal epidemics and periodic, unpredictable pandemics. Hemagglutinin (HA), the influenza surface glycoprotein, mediates viral entry into the cell and plays a key role during infection. HA is the major viral neutralizing antibody target and the most important antigen in influenza vaccines. Neuraminidase (NA), another viral surface protein, is also critical for viral infection during viral release from cells and a potential important additional antigen in future influenza vaccines.

**METHODS**

Alphavirus-based self-amplifying mRNA (sa-mRNA) encapsulated within a lipid nanoparticle (LNP) has been developed as a novel vaccine platform technology. We evaluated and selected bicistronic approaches for co-expression of HA and NA antigens from single sa-mRNA.

**RESULTS**

In vitro analysis showed that sa-mRNA bicistronic HA-NA led to robust expression of both HA and NA proteins in transfected cells. Mouse immunogenicity studies demonstrated that the sa-mRNA HA-NA vaccines raised strong anti-HA and anti-NA neutralizing antibody responses. Sa-mRNA HA-NA vaccines also elicited a Th1-dominant antigen specific CD4+ T cell responses and robust and broad CD8+ T cell response to both HA and NA.

**CONCLUSIONS**

Sa-mRNA HA-NA vaccines raised additional robust anti-NA antibody response and cell mediated immune response suggesting the new generation of vaccine may be more effective against influenza infection in humans than inactivated influenza vaccine.
Ensovibep, a potential antiviral COVID-19 treatment, is safe and well tolerated in healthy volunteers: preliminary safety and PK results from a phase 1, multi-part, ascending, single-dose study

**Topic:** Human influenza, RSV disease and COVID-19 challenge studies

Marianne Soergel (1), Stacy Gladman (2), Akeep Pun (2), Elena Hernandez (1), Nina Stojcheva (1), Tony Lockett (3), Pierre Fustier (1), Christof Zitt (1), Carine Marchand (1), Vaia Stavropoulou (1), Philippe Legenne (1), Malcolm Boyce (2)


**BACKGROUND**

Antiviral treatment options for early COVID-19 are needed in order to prevent disease progression in patients at risk. Ensovibep is a multi-domain DARPin® molecule that includes three different paratopes to bind to the same epitope of the receptor binding domain (RBD) of the SARS-CoV-2 spike protein, and two human serum albumin binding DARPin® domains for half-life extension. Ensovibep’s potential benefits include high affinity and strong binding to the viral S-protein, and low likelihood of ‘escape’ RBD mutations, due to its unique structure. We present preliminary safety, tolerability, and pharmacokinetic data of ensovibep.

**METHODS**

This is an ongoing multi-part, dose escalation study in healthy volunteers. In the completed Part A (double-blind, randomized, first-in-human, placebo-controlled), 23 subjects aged 20–64 years were enrolled as three sequential cohorts receiving 3 mg/kg (n=6 active), 9 mg/kg (n=6 active) or 20 mg/kg (n=5 active) ensovibep by intravenous (IV) infusion over 60 minutes. In the ongoing Part B (open-label), 8 subjects, aged 22–61 years, were enrolled as two sequential cohorts receiving 225 mg (n=4 active) or 600 mg (n=4 active) ensovibep by IV bolus injection. Safety and tolerability for both study parts assessed adverse events (AE), vital signs, 12-lead electrocardiogram (ECG), physical examination, laboratory safety tests and local infusion site tolerability assessments (up to Day 100 in Part A and Day 29 for Part B). Preliminary pharmacokinetics were also assessed.

**RESULTS**

Ensovibep showed a favorable safety profile in all subjects. No serious adverse events were reported, and all treatment emergent AEs (TEAEs) were mild (65%) or moderate (35%) in intensity. In Part A, 10 subjects reported TEAEs (4 subjects on placebo, 1 on 3 mg/kg ensovibep, 1 on 9 mg/kg ensovibep, and 4 on 20 mg/kg ensovibep). Overall, four TEAEs reported by 3 subjects were considered possibly related to treatment, including 3 mild events (erythema or bruise at infusion site; pain left arm) and one event of moderate hypersensitivity vasculitis in the 20 mg/kg cohort, with onset on Day 21, resolved by Day 38.

In Part B, 3 subjects reported TEAEs (1 on 225 mg; 2 on 600 mg) with 4 TEAEs possibly related to treatment (2 mild AEs of infusion site tenderness, and 1 subject with 2 moderate AEs of nausea and diarrhea).

There were no clinically significant findings from physical examination, vital signs, safety ECGs or changes in laboratory variables in either study part. Preliminary pharmacokinetic results indicate a half-life of around two-to-three weeks.

**CONCLUSION**

Single doses of ensovibep up to 20 mg/kg, administered intravenously (either over 60 min or by bolus) were safe, well tolerated and showed favorable pharmacokinetics. These findings support ongoing and future evaluation of ensovibep as potential COVID-19 treatment.
Intranasal M2SR (M2-deficient single replication) live H3N2 influenza investigational vaccine induces HAI responses against drifted influenza strains, as well as T cell responses, mucosal and serum IgA in serosusceptible adults

**Topic:** Human influenza, RSV disease and COVID-19 challenge studies

**Joseph Eiden (1), Ruth Ellis (2), Carlos Fierro (3), Howard Schwartz (4), Kimberly Ellis (5), Roger Aitchison (6), David Marshall (1), Yasuko Hatta (1), Renee Herber (1), Pamuk Bilsel (2)

1. FluGen, United States of America;
2. Biologics Consulting, United States of America;
3. Johnson County Clin-Trials, United States of America;
4. Research Centers of America, United States of America;
5. Alliance for Multispecialty Research, United States of America;
6. North Rim Consulting, United States of America

**Background**

In a previous human clinical trial (Eiden et al. JID 2021), $10^8$ TCID$_{50}$ (tissue-culture infectious dose) of H3N2 (A/Brisbane/10/2007) M2SR protected against influenza drifted strain challenge amongst a subset of subjects who responded to vaccination with a serum MNT (microneutralization) titer increase $>2$-fold, even though only 38% of the subset had $>4$-fold HAI response against the vaccine strain. In this subsequent phase 1b clinical trial (NCT03995554), higher doses of M2SR were evaluated for increased immune responses against vaccine and drifted influenza strains.

**Methods**

Safety and breadth of immunity were assessed after one and two dose intranasal administration of $10^8$ A/Brisbane/10/2007 or $10^5$, $10^6$ or $10^7$ doses of A/Singapore/INFIMH-16-0019/2016 H3N2 M2SR vaccines in a double-blind, randomized, placebo (saline)-controlled study conducted with 182 adult (18-49 years of age) volunteers.

**Results**

All dose levels of vaccine were well-tolerated without any safety concerns. Mucosal and serum IgA responses as well as T cell responses were highest for the $10^7$ dose of M2SR, with an additional, smaller increase after second dose (Figures 1 and 2). Serum HAI increases $>4$-fold were observed against the vaccine strain for 0%, 27.5% and 71% of subjects after first dose of placebo, $10^5$ or $10^7$ M2SR ($p<0.001$), respectively. Increases in HAI titers were also noted against highly diverse H3N2 strains (Figure 3).

**Conclusions**

Compared to the $10^8$ dose that protected against drifted strain challenge in a prior study, a single intranasal dose of $10^7$ M2SR stimulated cellular immune responses and significantly increased mucosal and humoral IgA responses among serosusceptible adults. The breadth of the immune responses and the stimulation of serum HAI titers (the recognized surrogate of protection for inactivated, intramuscular influenza vaccines) indicates the potential for M2SR to protect against infection and disease by matched as well as drifted influenza strains and supports additional clinical trials with multivalent M2SR.

![Graph showing mucosal and serum IgA responses](Image)

*Figure 1. Mucosal and serum IgA responses*
Figure 2. Influenza specific T cell responses

Figure 3. HAI responses against matched and drifted H3N2 strains
The Safety, Immunogenicity, and Efficacy of NVX-CoV2373, a COVID-19 Vaccine, When Co-administered With Seasonal Influenza Vaccines

Topic: Strategies for future Influenza vaccination

Seth Toback (1), Eva Galizia (2), Catherine Cosgrove (2), James Galloway (3), Anna L. Goodman (4), Pauline A. Swift (5), Fiona Burns (6), Angela M. Minassian (7), Paul T. Heath (2)

1: Novavax, United States of America;
2: St. George’s University of London, UK;
3: Kings College London, UK;
4: Guy’s and St Thomas’ NHS Foundation Trust, UK;
5: Epsom and St. Helier University Hospitals NHS Trust, UK;
6: University College London, and Royal Free London NHS Foundation Trust, UK;
7: University of Oxford and Oxford Health NHS Foundation Trust, UK

BACKGROUND

When two vaccines are co-administered, immune interference is often a concern. Current guidance is to separate administration of COVID-19 vaccines and seasonal influenza vaccines by 7 to 14 days, requiring an additional visit. The safety, immunogenicity, and efficacy of co-administration of these two vaccines has not been previously described.

METHODS

A sub-study investigating the co-administration of influenza vaccine was conducted as part of the pivotal phase 3 randomized trial of NVX-CoV2373 conducted in the United Kingdom. The main study enrolled 15,187 participants between September and November 2020. At 4 study locations, the first approximate 400 participants meeting main study entry criteria and with no contraindications to influenza vaccination were invited to join the sub-study. After randomization in a 1:1 ratio to receive NVX-CoV2373 (n=2317) or placebo (n=2314), sub-study participants received a licensed, open-label influenza vaccine with dose one of NVX-CoV2373. An adjuvanted trivalent or unadjuvanted quadrivalent influenza vaccine was given to those ≥65 and 18 to 64 years of age, respectively. Reactogenicity was evaluated via electronic diary for 7 days post-vaccination in the sub-study and a reactogenicity cohort from the main study. Unsolicited adverse events (AEs), medically-attended AEs (MAAEs), and serious AEs (SAEs) were collected in all participants. Influenza hemagglutination inhibition (HAI) and SARS-CoV-2 anti-spike IgG assays were performed in the sub-study and an immunogenicity cohort from the main study. Vaccine efficacy against PCR-confirmed, symptomatic COVID-19 was calculated. Non-randomized comparisons were made between influenza sub-study participants and main study reactogenicity and immunogenicity cohorts.

RESULTS

Sub-study participants were younger, more racially diverse, and had fewer comorbid conditions than main study participants. Reactogenicity events more common in the co-administration group included tenderness (70.1% vs 57.6%) or pain (39.7% vs 29.3%) at injection site, fatigue (27.7% vs 19.4%), and muscle pain (28.3% vs 21.4%). Rates of unsolicited AEs, MAAEs, and SAEs were low and balanced between the two groups. Co-administration resulted in no change to the influenza vaccine HAI response, while a reduction in geometric mean ELISA units in the anti-spike IgG assay was noted; co-administration: 31,236 (95% CI: 26,296, 37,105) vs NVX-CoV2373 alone: 46,678 (95% CI: 40,352, 49,468). In sub-study participants 18 to 64 years old, there was one case of PCR-confirmed, symptomatic COVID-19 with onset at least 7 days after the second dose among vaccine recipients and eight cases among placebo recipients; vaccine efficacy in the per-protocol sub-study was 87.5% (95% CI: 0.2, 98.4) while efficacy in the main study was 89.4% (95% CI: 79.7, 95.5).

CONCLUSION

This is the first study to investigate the co-administration of a COVID-19 vaccine with seasonal influenza vaccines. The results suggest concomitant vaccination may be a viable immunization strategy to help facilitate the uptake of both influenza vaccines and COVID-19 vaccines.
INFLUENCE OF ADJUVANTS AFTER INFLUENZA VACCINATION IN THE IMMUNODOMINANCE HIERARCHY

**Topic:** Strategies for future Influenza vaccination

Laura Sanchez-de Prada (1), Iván Sanz Muñoz (1), Raúl Ortiz de Lejarazu (1), Jose María Eiros-Bouza (1), Adolfo García-Sastre (2), Teresa Aydillo-Gómez (2)

1: National Influenza Center of Valladolid, Spain;
2: Icahn School of Medicine, Global Health and Emerging Pathogens Institute, Mount Sinai Hospital, NYC, USA

**BACKGROUND**

Hemagglutination inhibition assay (HAI) has been traditionally used to determine protection after influenza vaccination. HAI specifically measures the presence of antibodies (abs) against the head of hemagglutinin (HA), which is the main target of influenza vaccination. Classically 5 highly variable antigenic sites (Sb, Sa, Cb, Ca1, Ca2) were defined as immunodominant over other regions. The objective of our study is to determine differences in the immunodominance hierarchy triggered by quadrivalent (QIV) and adjuvanted trivalent influenza vaccines (ATIV).

**MATERIALS/METHODS**

Prospective study was performed in 162 individuals who received QIV (n=46, 52.3 years) or ATIV (n=116, 78.6 years) during 2018/2019 influenza season. Sera were obtained before and 28 days post-vaccination. Five recombinant viruses were generated: H1ΔSa, H1ΔSb, H1ΔCa1, H1ΔCa2, H1ΔCb, where the classically defined H1 antigenic sites had been partially substituted with heterologous antigenic sites from either H5 or H13 HAs. These viruses contained an encoded HA from A/Michigan/45/2015 strain (WT-H1), while the seven remaining segments were encoded by A/Puerto Rico/8/1934 (PR8). Abs were measured by HAI against this panel of viruses. To represent immunodominance, two parameters were created: Reduction rate and Dominance index (DI). First one is defined as “(FI mutant virus/FI WT H1) x 100,” where Fold induction (FI) is calculated dividing post-vaccine and pre-vaccine geometric mean titers (GMT). Second one represents the reduction of HAI titers against mutant viruses before and after vaccination compared to WT-H1.

**RESULTS**

In the QIV, significant differences were found in H1ΔSb and H1ΔCa2 who reached 46.4% and 64.6% of the FI reached by the WT-H1. With ATIV, significantly lower rates were achieved with values of 48% for H1ΔSb, followed by H1ΔCa2 (74.2%), H1ΔCb (85.1%), H1ΔSa (87.2%) and H1ΔCa1 (89.2%) (ANOVA, p<0.05). The DI showed how with QIV responses after vaccination are mainly generated against dominant antigenic sites (Sa and Ca2). Whereas, with ATIV, response is created against all antigenic sites, still with a predominance of Sa followed by Ca2.

**CONCLUSIONS**

Our results confirm that humoral response is mainly triggered by antigenic sites surrounding the Receptor Binding Site (Sa and Ca2). Adjuvants increase the breadth of protection providing a more uniform response and not only towards the immunodominant antigenic sites. However, age also could be a factor contributing to that effect.
Quantification of correctly-folded chimeric hemagglutinin in split inactivated influenza A and B virus vaccines

**Topic:** Strategies for future Influenza vaccination

**Eduard Puente-Massaguer, Guha Arunkumar, Madhumathi Loganathan, Florian Krammer**

Icahn School of Medicine at Mount Sinai, United States of America

**INTRODUCTION**

The stalk domain of the hemagglutinin (HA) has become the prime target for universal influenza virus vaccine development in the last few years. Unlike the HA head domain, the immunosubdominant stalk domain is conserved to a higher level within each influenza virus group. Sequential vaccination with chimeric HA viruses consisting of the same HA stalk and exotic head domains has been proven to re-direct the immune response towards the HA stalk. This vaccination concept puts the basis for the development of more broadly cross-protective vaccines that are less affected by antigenic shift and drift, one of the main drawbacks from currently marketed influenza vaccines. In this sense, there is a need to develop quantification methods that measure the correct stalk conformation of chimeric HA-based virus vaccines.

**METHODS**

A capture enzyme-linked immunosorbent assay (ELISA) is developed and employed to detect and quantitatively measure the HA with conformationally intact stalk epitopes. Different monoclonal antibodies (mAbs) that bind to epitopes on the head and stalk domains of different HA subtypes are tested. Specifically, the binding of 1A7 (anti H8 head), 1H4 (anti H5 head), 1G4 (anti H4 head), 6F6, 3G11, 6G8 (anti H15 head), SC5, CR8033, CR8059 (anti pan-influenza B head), 9H10 (anti pan-group 2 stalk), 4G12, 4C10, 2G4 (anti pan-influenza B stalk), and CR9114 (anti pan-HA stalk) is tested against split inactivated ch18/1N1, ch5/1N1 (group 1), ch4/3N2, ch15/3N2 (group 2), mH13BP2Brisbane and mH5BPPhuket (influenza B) chimeric HA viruses. A pair of mAbs consisting of one mAb that binds the stalk domain and one mAb that targets the head domain of the HA are selected for each chimeric HA virus. A recombinant chimeric HA protein standard of known concentration is also included in the assay for absolute HA quantification.

**RESULTS**

All different mAbs selected bind to the split inactivated chimeric HA viruses, and no competition is detected between anti-stalk and anti-head mAbs for binding to their epitope. The selection of the mAb pair for the capture ELISA of each virus is performed according to the maximum binding signal measured for each mAb, and the comparison of each mAb binding signal and the binding signal of the corresponding recombinant HA protein standard. In these conditions, the mAb pairs for the capture ELISA of each virus are defined as: 1A7 & CR9114 (ch18/1N1), 1H4 & CR9114 (ch5/1N1), 3G11 & CR9114 (ch15/3N2), 1G4 & CR9114 (ch4/3N2), CR8033 & 4C10 (mH13BP2Brisbane and mH5BPPhuket). This method is successfully applied for the quantification of correctly-folded HA in the different split inactivated chimeric HA virus vaccines.

**CONCLUSIONS**

A capture ELISA to quantitatively measure the concentration of correctly-folded HA in vaccine preparations is developed. This assay could be used in the development of broadly protective/universal influenza virus vaccines and could be established as a potency assay for these stalk-based vaccine constructs.
Novel immunogen design elicits increased protection against avian H7N9 influenza that is associated with mobilization of seasonal influenza T cell memory

**Topic:** Strategies for future Influenza vaccination

Mayaya Grizotte-Lake (1), Hyeseon Jang (2), Andres Gutierrez (1), Christine Boyle (1), Lauren Myers (1), Nese Kurt Yilmaz (3), Celia Schiffer (3), Ted Ross (2,4), Anne De Groot (1,2), Lenny Moise (1,2)

1. EpiVax, Inc., Providence, RI, United States of America; 2. Center for Vaccines and Immunology, University of Georgia, Athens, GA, United States of America; 3. Department of Biochemistry and Molecular Pharmacology, UMass Medical School, Worcester, MA, USA; 4. Department of Infectious Diseases, University of Georgia, Athens, GA, USA

**BACKGROUND**
Cross-conserved hemagglutinin (HA)-specific CD4+ T cell epitopes support protective B cell responses against seasonal influenza. However, in the case of avian H7N9, which poses a pandemic threat, HA elicits only weak neutralizing antibody responses in infection and vaccination without adjuvant. Strategies to optimize H7N9 HA immunogenicity are therefore critical for pandemic preparedness. Because cross-conserved T cell epitopes augment seasonal influenza, we hypothesized that an immune-engineered H7N9 HA incorporating broadly reactive H3N2 HA-specific memory CD4+ T cell epitopes would boost protective antibody responses and increase protection.

**METHODS**
We designed and produced two optimized H7N9 HA constructs: OPT1 (three amino acid substitutions replace a reported regulatory T cell (Treg) epitope with a highly conserved and broadly reactive CD4+ T cell epitope from H3-HA) and OPT2 (integrates six H3-HA CD4+ T cell epitopes, one Treg epitope removed). Structure modeling and molecular dynamics simulations of the engineered constructs were performed to assess perturbation of antibody target structures and predict folding stability. Vaccination studies were carried out in HLA-DR3 transgenic mice pre-immune to H3N2 (A/Hong Kong/4108/2014). Mice were primed and boosted IM with wildtype or engineered H7N9 HA without adjuvant eight weeks post-H3N2 exposure. T cell phenotypes, total IgG, and HAI titers to H7 and H3 and H7 were monitored. Mice were challenged IN with H7N9 virus (A/Anhui/1/2013) four weeks following boost and followed for weight loss and survival.

**RESULTS**
Vaccination of H3N2 pre-immune mice with OPTimized H7 immunogens expanded TCM (CD14+CD62L+) and TEM (CD14+CD62L-) levels over wildtype H7. A higher increase was induced by the OPT2 vaccine, which is consistent with its higher H3 epitope load and ability to recruit more H3-HA-specific CD4+ T cells than OPT1. A similar increase in Tfh (CXCR5+PD1+ICOS+Bcl6+) in animals that received OPTimized H7 immunogens was observed, with no significant difference between OPT1 and OPT2. Anti-H7 IgG responses are found after prime-boost with OPT1 and OPT2 while no antibody response to wildtype vaccine is found before a third immunization. Both OPTimized vaccines completely protected against lethal H7N9 virus infection while in contrast, wildtype vaccinated animals had a survival rate that was similar to unvaccinated pre-immune controls. OPT1 and OPT2 lowered average weight loss post-infection with OPT2 animals maintaining >95% of their weight.

**CONCLUSIONS**
We find increased vaccine-induced protection against a pandemic influenza strain is associated with enhanced T cell immunity elicited by a novel immunogen design strategy that harnesses seasonal influenza CD4+ T cell memory. Structure-guided T cell epitope modification may also be useful to develop more effective vaccines against seasonal influenza strains.
Pre-existing antibodies directed against a heterologous tetramerizing domain boost the immune response against artificially stabilized soluble tetrameric influenza neuraminidase

**Topic:** Strategies for future Influenza vaccination

**João Paulo Portela Catani (1,2), Emma R. Jelb (1,2,4), Tine Yvensbaert (1,2), Anouk Smet (1,2), Satyajit Ray (3), Lauret LaRue (3), Svetlana Stegalikina (3), Marie Banna (3), Thorsten U. Voegel (3), Xavier Saenens (1,2)**

1: VIB-UGent Center for Medical Biotechnology, VIB, Belgium; 2: Department of Biochemistry and Microbiology, Ghent University, Belgium; 3: Sanofi Pasteur, Research North America, Cambridge, Massachusetts, USA; 4: Current affiliation: Janssen Infectious Diseases and Vaccines, Janssen Research and Discovery, Beerse, Belgium

**BACKGROUND**

Neuraminidase (NA) is one of the major antigens present at the surface of influenza virions. NA acts in concert with hemagglutinin (HA) during the early stages of infection, enabling movement of virions through the decoy receptor and facilitating release of newly produced virions from infected cells. Despite being an independent correlate of protection, NA-inhibiting antibodies are absent or poorly induced by current influenza vaccines, which are primarily based on HA as the target immunogen. Supplementing inactivated influenza vaccines with NA could potentially improve their protective potential. Influenza NA is active only when assembled into a homo-tetramer which is promoted by its transmembrane domain. Soluble tetrameric NA can be obtained by the fusion of the catalytic NA head domain to a tetramerizing zipper such as tetrabrachion (Tet). Although Tet-stabilized NA is being explored as an NA inhibiting (NAI) antibody-inducing vaccine antigen, the immunogenicity of the Tet zipper and its impact on the immune response against the fused partner have not been evaluated.

**METHODS**

To evaluate the impact of pre-existing anti-Tet immunity on the response against NA, mice were immunized three times with Tet fused to human serum albumin domain III (tetHSA) and subsequently two times with tetNA. The induction of NA-specific antibodies was quantified by ELISA and an enzyme-linked lectin assay (ELLA). In addition, to determine the role of anti-tetrabrachion antibodies on the induction of NA responses induced by immunization with tetNA, serum from tetHSA immunized mice was transferred to naïve mice that were subsequently immunized with tetNA. Finally, to understand the mechanism behind the observed increase of NAI titer in mice with anti-Tet immunoglobulins, FcεRIγ-/- mice, which lack the activating Fcg receptors, were immunized with tetHSA followed by tetNA and induction of anti-NA antibodies was determined.

**RESULTS**

The Tet domain is immunogenic and induces Tet-specific serum IgG titers. Pre-existing anti-Tet antibodies increase the anti-NA IgG and NAI titers upon tetNA immunization. The increase of NAI titers after anti-Tet serum transfer indicates that the mechanism of this enhanced anti-NA response can operate independently of a cellular response. The increased NAI titers induced upon vaccination with tetNA of mice with pre-existing anti-Tet antibodies, was observed also in FcεRIγ-/- mice, suggesting a mechanism which is independent of signaling of immune complexes through FcγRI, FcγRII, FcγRIII, and FcεRI.

**CONCLUSIONS**

An immune-enhancing effect on the induction of anti-NA antibody responses was observed after repeated Tet immunization followed by immunization with tetNA. These findings may have implications for the repeated use of vaccines in which heterologous oligomerizing zippers are used to stabilize a soluble oligomeric antigen.

![Figure 1](image.png)

*Figure 1: Immunization of mice with tetHSA increases the anti-NA response induced by a subsequent tetNA immunization.*
IL-6, IL-8 and IFN-β are elevated in individuals with long COVID

**Topic:** "Long COVID": post acute clinical sequelae of COVID-19

Jane Sinclair (1), Zhen Wei Marcus Tong (1), Ellesandra Noye (1), Keng Yih Chew (1), Matthew Trau (2), Alain Wuethrich (2), Corey Smith (3,4), Kirsten Short (1)

1: The University of Queensland, School of Chemistry and Molecular Biosciences, St Lucia, Australia; 2: The University of Queensland, Australian Institute for Bioengineering and Nanotechnology, St Lucia, QLD 4072, Australia; 3: QIMR Berghofer Centre for Immunotherapy and Vaccine Development and Translational and Human Immunology Laboratory, Department of Immunology, QIMR Berghofer Medical Research Institute, Herston, QLD 4006, Australia; 4: The University of Queensland, Faculty of Medicine, St Lucia, QLD 4072, Australia

**BACKGROUND**

Long COVID patients may experience a range of persistent, de novo and recurring symptoms affecting the respiratory, cardiovascular, musculoskeletal, and neurological systems >4 weeks post-infection. Emerging literature indicates that chronic inflammation drives long COVID in a subpopulation of recovered COVID-19 patients, while for others, disease symptoms are instead linked to SARS-CoV-2 induced organ damage, the nonspecific effects of hospitalisation and/or the adverse effects of medication/interventions applied during acute infection. This heterogeneous pathophysiology highlights the importance of determining in which patients anti-inflammatory therapies would be of clinical benefit.

**METHODS**

Serum was donated from Australians who have recovered from COVID-19, 2-4 months post-COVID-19 diagnosis. Long COVID was defined as the presence of persisting, recurring or de novo symptoms that could not be attributable to another diagnosis at the time of sera donation. BioLegend LEGENDplex Human Anti-Virus Response Panel (13-plex) was used to measure serum levels of IL-1β, IL-6, TNF-α, IP-10, IFN-α1, IL-8, IL-12p70, IFN-α2, IFN-α2/3, GM-CSF, IFN-β, IL-10, and IFN-γ.

**RESULTS**

Upon blood donation, 19/68 (27.9%) of patients who had recovered from COVID-19 reported persistent COVID-19 symptoms. Of the patients suffering from long COVID, 6 (31.6%) reported experiencing lethargy and fatigue, 4 (21.1%) cardiovascular problems, 4 (21.1%) respiratory problems, 3 (15.8%) altered sense of smell, 2 (10.5%) kidney problems, 1 (5.3%) elevated C-reactive protein, 1 (5.3%) brain fog, and 1 (5.3%) body ache, while 3 (15.8%) reported undisclosed persistent symptoms. Patients suffering from long COVID experienced significant elevations in serum IL-6 (p=0.0271), IL-8 (p=0.0441), and IFN-β (p=0.0005).

**CONCLUSIONS**

Cytokines IL-6, IL-8 and IFN-β were significantly increased in Australian patients suffering from long COVID in comparison to patients who had fully recovered from COVID-19. Characterising inflammation in long COVID patients is a vital step in aiding identification of those that would benefit from anti-inflammatory therapy.
Pulmonary function and quality of life recovery in COVID-19 pneumonia survivors

Topic: "Long COVID": post acute clinical sequelae of COVID-19

Edita Strumiliene (1,2), Tadas Zvirblis (3), Ligita Jancoriene (1,2)

1: Faculty of Medicine, Vilnius University, Lithuania;
2: Vilnius University Hospital Santaros Klinikos, Lithuania;
3: Institute of Mechanical Science, Vilnius Gediminas Technical University

BACKGROUND

It is already known, that clinical symptoms and residual lung damage in COVID-19 pneumonia survivors may persist for months. But the recovery period - restoration of lung function and health related quality of life (HR-QoL) - has still been poorly analyzed. We performed the analysis of these data at 6 months follow-up period.

METHODS

COVID-19 pneumonia patients were included, if they did not have chronic lung disease prior SARS-CoV-2 infection. Evaluation consisted of spirometry (FVC, FEV1, FEV1/FVC), lung volumes (TLC, VC, RV), diffusing capacity of lung for carbon monoxide (DLCO) testing and 36-item Short Form General Health Survey (SF-36). Radiological examination was also performed at the first visit – at 3 months after the discharge from hospital. The follow-up was continued at 6 months (second visit), if any radiological abnormalities in the lungs or impairment of lung function was detected.

RESULTS

112 consecutive patients were evaluated at 3 months after the discharge from hospital. 60 patients (53.6%) were continued to be monitored due to confirmed residual abnormalities.

The mean age was 57.1 years (31-80); 29 were men. Eleven patients (18%) were after moderate COVID-19, 28 (47%) after severe and 21 (35%) after critical disease.

Obstruction with reduced FEV1/FVC was not detected.

DLCO reduction at 3 months visit was observed in 29 patients (48.3%) and reduced to 13 patients (21.7%) at 6 months. Restriction was observed in 19 patients (31.7%) at first visit and was still observed in 14 (23.3%) at second visit. The statistically significant difference between disease severity groups was found in all these parameters at both visits (p<0.05), showing that the consequences has high correlation with the severity of the disease.

SF-36 scores demonstrated a reduction in health status across all domains at 3 and 6 months compared to healthy population in all patients with the improvement over time (data presented in Table1).

![Graph showing HR-QoL assessment](image)

Table1. HR-QoL assessment: the changes over time between disease severity groups and healthy population.

CONCLUSION

Our results indicate that impaired lung function and reduced HR-QoL status improves over time. But 6 months in COVID-19 pneumonia survivors might still be not enough for the full recovery.

**Topic:** "Long COVID": post acute clinical sequelae of COVID-19

Maxime Taquet

University of Oxford, United Kingdom
Induction of Broadly Cross-neutralizing Antibody Responses Against A(H3N2) and B Viruses: Results of a Phase 3 Trial of a Recombinant Quadriavalent Hemagglutinin Saponin-adjuvanted Nanoparticle Influenza Vaccine in Older Adults

**Topic:** Strategies for future RSV disease vaccination

**Vivek Shinde, Wayne Woo, Joyce Plested, Tim Vincent, Bin Zhou, Minghui Zhu, Chuan-Feng Shih, Shane Cloney-Clark, Rongman Cai, Gary Albert, Haixia Zhou, Michael Massare, Kathy Smith, Lou Fries, Gale Smith, Ik Sung Cho, Greg Glenn

Novavax, United States of America

**BACKGROUND**

There is an urgent public health need for more effective seasonal influenza vaccines for older adults, which can induce broadly cross-reactive antibodies and potent T-cell responses against antigenic drift variants, particularly A(H3N2) viruses, while avoiding egg-adaptive antigenic changes. We developed a recombinant quadrivalent hemagglutinin saponin-adjuvanted (Matrix-M) nanoparticle influenza vaccine (qNV; NanoFlu), and recently reported preliminary results of a phase 3 trial, wherein qNV demonstrated immunologic non-inferiority to an egg-derived quadrivalent inactivated influenza vaccine (IVIV; Fluzone Quadrivalent) using an egg-based hemagglutination-inhibiting (HAI) antibody assay. Corresponding post-vaccination (Day 28) wild-type (i.e., without egg adaptations) HAI antibody responses induced by qNV were significantly higher than IVIV against four vaccine-homologous strains (24-65% increased) and six heterologous A(H3N2) strains (34-69% increased) representing multiple distinct clades/subclades. qNV also induced potent post-vaccination (Day 7) polyfunctional antigen-specific effector CD4+ T-cell responses, which were 126-189% higher compared to IVIV. We now report post-vaccination wild-type microneutralization (wt-MN) antibody responses and long-term safety data.

**METHODS**

In this phase 3 trial, we randomized 2,652 participants aged ≥65 years 1:1 to receive a single intramuscular dose of qNV or IVIV and assessed pre-/post-vaccination strain-specific wt-MN antibody responses (Days 0, 28) in a randomly-selected subset of participants against a panel of wild-type vaccine-homologous viruses, and wild-type antigenically drifted A(H3N2) and B viruses. Long-term safety outcomes, including medically attended adverse events (MAAEs), serious adverse events (SAEs), and potential immune-mediated adverse events of special interest (AESIs) were assessed through Day 364.

**RESULTS**

Post-vaccination wt-MN antibody responses induced by qNV were significantly higher than IVIV against all four vaccine-homologous strains: A/Brisbane[H1N1](12% higher; p<0.04), A/Kansas[H3N2](78% higher; p<0.001), B/Maryland[B-Victoria](52% higher; p<0.001), B/Phuket[B-Yamagata](38% higher; p<0.001), and against 4 of 5 drifted A(H3N2) or B strains: A/Hong Kong[H3N2](61% higher; p<0.001), A/South Australia[H3N2](59% higher; p<0.001), A/Wisconsin[H3N2](11% higher; p=0.07); or B/Washington[B-Victoria](67% higher; p<0.001). Through Day 364, the frequency of MAAEs were comparable in qNV vs IVIV (26.5% vs 26.8%). SAEs and AESIs were infrequent in both groups (6.1% vs 5.9%; and 3.2% vs 3.7%).

**CONCLUSIONS**

qNV had a long-term safety profile comparable to IVIV, and induced enhanced wild-type neutralizing and broadly cross-neutralizing antibody responses as compared to IVIV against all 4 vaccine-homologous viruses, and also antigenically drifted A/H3N2 and B viruses. Using wild-type neutralizing antibody responses as a confirmatory functional antibody assay, we corroborated previously reported broadly cross-reactive wild-type HAI antibody responses. qNV produced qualitatively and quantitatively enhanced immune responses, which may address several critical challenges confronting current egg-derived seasonal influenza vaccines, especially in the older adult population.
Safety and Tolerability of an Ad26.RSV.preF-based Vaccine in a Randomized, Double-blind, Placebo-controlled, Phase 2b Study in Adults Aged ≥65 Years

**Topic:** Strategies for future RSV disease vaccination

Stephan Bart (1), Kristi Williams (2), Efthymios Gynopoulos (3), Ann R Falsey (4), John Ervin (5), Arangassery Rosemary Bastian (6), Joris Menten (3), Els De Paepe (3), Hilde de Boer (7), Jouke Vandenberghe (3), Eric Chan (8), Jerald Sado (6), Macaya Douoguih (6), Benoit Callendret (6), Esther Heijnen (6), Christy Comeaux (6)

1: Optimal Research, LLC/Synexus Clinical Research/AES, Woodstock, MD, USA; 2: Janssen Research and Development, Spring House, PA, USA; 3: Janssen Infectious Diseases, Beerse, Belgium; 4: University of Rochester School of Medicine, Rochester, NY, USA; 5: AMR Kansas City, Kansas City, MO, USA; 6: Janssen Vaccines & Prevention BV, Leiden, The Netherlands; 7: Janssen-Cilag, Tilburg, The Netherlands; 8: Janssen Global Services, LLC, Raritan, NJ, USA

**BACKGROUND**

Respiratory syncytial virus (RSV) is recognized as a major cause of respiratory disease in older adults; however, there is currently no approved prophylactic vaccine. Ad26.RSV.preF is a recombinant, replication-incompetent, human adenovirus serotype 26 (Ad26) vectored RSV vaccine, encoding a conformation-stabilized prefusion RSV F (preF) protein. Here, we report the safety and reactogenicity of an Ad26.RSV.preF-based vaccine in adults aged ≥65 years in a Phase 2b proof-of-concept trial.

**METHODS**

CYPRESS (NCT03982193) is a multicenter, randomized, double-blind, placebo-controlled, Phase 2b trial. Adults ≥65 years of age in stable health were randomized 1:1 prior to the RSV season to receive Ad26.RSV.preF-based vaccine or placebo. Solicited adverse events (AEs; fatigue, headache, nausea, myalgia, fever, injection site reactions) and unsolicited AEs were assessed from time of vaccination (Day 1) to Day 8 and Day 29, respectively, in a safety subset of 695 participants (vaccine, n=348; placebo, n=347). Serious AEs (SAEs) were collected in all participants until the end of the RSV season (primary endpoint) or 6 months post-vaccination, whichever occurred later.

**RESULTS**

A total of 5782 participants were randomized and received Ad26.RSV.preF or placebo (n=2891 in each group). Demographics and baseline clinical characteristics were similar between the study groups (92.5% white, 57.7% female, median age 71 years). In the safety subset, the frequency of solicited AEs and Grade ≥3 solicited AEs was 51.4% and 3.2% in the vaccine group and 20.2% and 0.6% in the placebo group, respectively. In the vaccine group, the most frequent solicited systemic AEs were fatigue, myalgia, and headache, and the most frequent solicited local AE was pain/tenderness. The rates of unsolicited AEs and Grade ≥3 unsolicited AEs were similar in both groups (vaccine, 16.7% and 1.7%; placebo, 14.4% and 1.4%). In the overall study population, the rate of SAEs was similar between groups (vaccine, 4.6%; placebo, 4.7%), and none were found to be related to the vaccine. The incidence of AEs leading to study discontinuation was similar between groups (vaccine, 0.3%; placebo, 0.5%).

**CONCLUSIONS**

Ad26.RSV.preF-based vaccine was safe and well tolerated in adults aged ≥65 years.

Study Group CYPRESS

Keywords: Respiratory syncytial virus, viral vaccines, adult immunization
**Strategies for future RSV disease vaccination**

Christopher A. Comeaux (1), Stephan Bart (2), Arangassery Rosemary Bastian (1), Efi Gymnopoulou (3), Els De Paepe (3), Ray van Heesbeen (1), Esther Heijnen (1), Benoit Callendret (1), Jerald Sadoff (1)

1: Janssen Vaccines & Prevention B.V., Leiden, The Netherlands; 2: Optimal Research, LLC/Synexus Clinical Research/AES, Woodstock, MD, USA; 3: Janssen Infectious Diseases BV, Beerse, Belgium

**Background**

Despite the high disease burden of respiratory syncytial virus (RSV) in older (>60 years) and vulnerable adults, there is no licensed prophylactic RSV vaccine. Ad26 RSV preF is a recombinant, replication-incompetent, adenovirus serotype 26-based RSV vaccine encoding a conformation-stabilized prefusion RSV F (pref) protein. A human challenge study of Ad26 RSV preF showed promising efficacy results. The objective of this study was to evaluate whether a single immunization using Ad26 RSV preF in combination with recombinant RSV preF protein might further enhance the RSV-specific humoral immune response.

**Methods**

In this adaptive, randomized, double-blind placebo-controlled Phase 1/2a study (VAC18193RSV1004; NCT03502707), we evaluated the safety and immunogenicity of Ad26 RSV preF, RSV preF protein, or combinations (mix regimens) of Ad26 RSV preF and RSV preF protein in adults aged >60 years. Two dose levels of each component were used: Ad26 RSV preF (5x10^9 viral particles [vp], 1x10^11 vp) and RSV preF (50 μg, 150 μg). Three sequential cohorts (n=657) were enrolled: an initial safety cohort (Cohort 1) to assess safety of RSV preF protein alone and in combination with Ad26 RSV preF; a regimen selection cohort (Cohort 2) to assess the safety and immunogenicity of Ad26 RSV preF and mix regimens (dosing at day 1); and an expanded safety cohort (Cohort 3) with the selected regimen (dosing at day 1). A primary analysis was conducted at 28 days post-dose 1 (Cohort 2) for regimen selection for late-stage development; humoral responses were assessed by virus neutralization assay (VNA) and preF binding antibody ELISA, and cellular responses using RSV-F-specific INF-γ ELISPOT.

**Results**

Overall, 352 participants were enrolled and vaccinated in Cohorts 1 and 2. All vaccine combinations were safe and well tolerated, with no differences observed between mix regimens or mix regimens versus Ad26 RSV preF alone. In Cohort 2, all mix regimens substantially increased neutralizing antibody (VNA) titers and showed a 1.8-3.5-fold increase compared to Ad26 RSV preF alone and a 5.5-10.3-fold increase compared to baseline 28 days post-vaccination. Similar results were observed for other humoral responses measured, including preF binding antibodies. A favorable ratio of binding to neutralizing antibodies and similar RSV-F-specific INF-γ ELISPOT values were maintained across all mix regimens compared to Ad26 RSV preF alone. The data suggested no impact of pre-existing vaccine-induced humoral and cellular immune responses measured for any of the regimens tested.

**Conclusions**

All Ad26 RSV preF- and RSV preF-based regimens were safe and well tolerated. Immunogenicity analyses suggest that the optimal dosing regimen is a mix of high dose Ad26 RSV preF, which elicits a strong T-cell response, with high dose RSV preF protein, which increases humoral immune responses.

**References**


**Keywords** Respiratory syncytial virus, viral vaccines, adult immunization
**An Ad26.RSV.preF-based Vaccine is Effective for Prevention of RSV-mediated Lower Respiratory Tract Disease and Reduces Symptom Severity in Vaccine Recipients With RSV Infection: A Phase 2b Study in Older Adults**

**Topic:** Strategies for future RSV disease vaccination

Christy Comeaux (1), Ann R Falsey (2), Kristi Williams (3), Eli Gynnapoulou (4), Stephan Bart (5), John Envin (6), Arangassery Rosemary Bastian (1), Jordis Menten (4), Ellis De Paepe (4), Hilde de Boer (7), Sjoekje Vandenbergh (4), Eric Chan (8), Jerold Sadoff (1), Macaya Deugulhi (2), Benoit Callendret (1), Esther Heijnen (1)

1: Janssen Vaccines & Prevention BV, Leiden, The Netherlands; 2: University of Rochester School of Medicine, Rochester, NY, United States of America; 3: Janssen Infectious Diseases, Beerse, Belgium; 4: Janssen Research and Development, Spring House, PA, USA; 5: Optimal Research, LLC/Synexus Clinical Research/AES, Woodstock, MD, USA; 6: AMR Kansas City, Kansas City, MO, USA; 7: Janssen-Cilag, Tilburg, The Netherlands; 8: Janssen Global Services, LLC, Raritan, NJ, USA

**BACKGROUND**

Respiratory syncytial virus (RSV) is an important cause of lower respiratory tract disease (LRTD) in older adults. Currently, no licensed vaccine for the prevention of RSV is available. Ad26-RSV.preF is a recombinant, replication-incompetent, human adenovirus serotype 26 (Ad26) vectored RSV vaccine, encoding a conformation-stabilized prefusion RSV F (preF) protein. In this Phase 2b proof-of-concept trial, we report the vaccine efficacy of Ad26-RSV-preF in combination with recombinant RSV preF protein for the prevention of RSV-mediated LRTD in adults aged ≥65 years.

**METHODS**

CYPRESS (NCT03982193) is a randomized, double-blind, placebo-controlled, Phase 2b trial. Adults aged ≥65 years were randomized 1:1 prior to the RSV season to receive an Ad26-RSV.preF-based regimen or placebo. The primary endpoint was the first occurrence of RT-PCR-confirmed RSV-mediated LRTD in the first RSV season according to any of 3 case definitions: (1) ≥3 symptoms of lower respiratory tract infection (LR1), (2) ≥2 symptoms of LR1 or (3) ≥2 symptoms of LR1 or ≥1 symptom of LR1 with ≥1 systemic symptom. The secondary endpoint was the first occurrence of any RT-PCR-confirmed RSV-mediated acute respiratory infection (ARI). ARI symptoms were collected using an RSV-specific patient-reported Respiratory Infection Intensity and Impact Questionnaire (RiiQ; daily during the ARI episode) and/or by clinician assessment (ARI Day 3, 4, or 5). An independent clinical evaluation committee (CEC) assessed participant- and clinician-reported data for participants with RSV-positive ARIs to determine the location (upper or lower respiratory tract) and severity of the ARI. RiiQ scores and time to return to usual health (Patient Global Impression Return to Usual Health Question) were compared between participants with RSV ARIs in the vaccine versus placebo groups.

**RESULTS**

A total of 5782 participants (2891 in each study group) received study treatment (92.9% white, 57.7% female, median age 71 years). Vaccine efficacy for LRTD case definitions 1, 2, and 3 was 89.2% (95% CI 52.2-92.9%), 76.3% (95% CI 60.0-84.2%), and 69.8% (63.7-84.7%), respectively (all P values <0.001).

Efficacy was 69.8% (95% CI 42.7-85.1%) for the first occurrence of any RSV-mediated ARI and 67.9% (50.1-88.5%) for CEC-assessed RSV-positive moderate/severe LRTD. Median area under the curve (AUC) for total RiiQ scores was lower (indicating less severe symptoms) in participants in the vaccine group with RSV ARI (n=13; median AUC [Q1; Q3]: 39 [11; 74]) than in the placebo group (n=41; 128 [58; 242]). Time to return to usual health after RSV ARI was faster in the vaccine group than in the placebo group (median, 19 vs 30 days; HR [95% CI], 2.812 [1.006-7.86]).

**CONCLUSIONS**

Ad26-RSV.preF-based vaccine was highly effective for the prevention of RSV-mediated LRTD during the first RSV season in adults aged ≥65 years. Vaccinated participants who experienced RSV-mediated ARIs had milder symptoms and faster return to usual health than the placebo group.

**Study Group CYPRESS**

**Keywords:** Respiratory syncytial virus; viral vaccines; adult immunization
Public health benefits of switching into a recombinant quadrivalent vaccine in the Spanish Murcia and Valencia regions the recommended adult population (18+) for influenza seasonal vaccination.

**Topic:** Why influenza and RSV disease are a priority for policy makers

**Georgina Drago Manchón (1), Juan Luis López-Belmonte (2), Hélène Bricout (3), Caroline de Courville (3)**

1: Sanofi Pasteur, Barcelona, Spain;  
2: Sanofi Pasteur, Madrid, Spain;  
3: Sanofi Pasteur Lyon, France

**BACKGROUND**

To assess the clinical impact and healthcare consumption differences associated with the switch to a recombinant quadrivalent influenza vaccine (RIV4) in the recommended adults’ population (18+) from the regions of Murcia and Valencia community (Spain).

**METHODS**

A decision tree model was used to compare the burden of influenza disease in adults aged 18 years or older vaccinated with RIV4 vs. SD-QIV [18-64 yo at risk] and aQIV [≥65 yo] from National Healthcare System perspective. The model predicts averted influenza cases, influenza-related general practitioner (GP) and emergency room (ER) visits, hospitalizations attributable to influenza and influenza-related deaths, within a one-year time horizon for these health outcomes.

The influenza attack rates were extracted from a systematic literature review providing age-stratified values (18-64 yo [4.4%] and 65 or older yo [7.2%]). The vaccine coverage rates (VCR) were from regional specific data; probabilities of GP and ER visits clinically diagnosed on influenza were based on Spanish data. Hospitalization data were estimated from a modelling study that assessed the excess cardiorespiratory hospitalization rate attributable to influenza during 9 influenza seasons in Spain (average of 2008/09 to 2017/18, excluding 2009/10 season). The mortality data correspond to the 6-season average of all cause excess mortality attributable to influenza estimated with the EuroMOMO model. All demographic inputs were for those of the two Spanish regions.

The relative efficacy of RIV4 vs. SD-QIV of 30% was based on a randomized controlled trial. In the absence of RCT data, the relative effectiveness of SD-QIV vs. aQIV was assumed to be 6% based on Spanish observational study.

**RESULTS**

In Murcia and Valencia Regions (Spain), switching from SD-QIV to RIV4 in at-risk adults aged 18 to 64 yo and from aQIV to RIV4 in those 65 or older, would avert 8,927 influenza cases (12%), with 104 fewer deaths (14%). It also allows a reduction of 3,999 GP and 220 ER visits and 760 (14%) cardiorespiratory hospitalizations.

**CONCLUSIONS**

A switch from current standard of care to RIV4 in Murcia and Valencia (Spain), is recommended for influenza vaccination population, would contribute reduce both, excess mortality and healthcare resources consumption.

*This work was funded by Sanofi Pasteur*
Open Science Practices Are Favored by Most Surveyed Influenza Researchers Despite A Lack of Perceived Peer Support

**Topic:** Why influenza and RSV disease are a priority for policy makers

**Kelly Sutherland, David Melior**

The Center for Open Science, United States of America

**BACKGROUND**

A lack of transparency is not just a crisis in the social and behavioral sciences — it is also an issue in microbiology (Schloss, 2017). While practices such as data sharing and preregistration have spread in some communities, they are not widespread in areas such as virology or pre-clinical influenza research. This has become especially evident as a result of the COVID-19 pandemic, which has served as a catalyst for the rapid adoption of practices such as pre-printing (Besançon et al., 2021). Prior to the pandemic, scientists reported that pressure to publish and selective reporting often contributed to issues with reproducibility (Baker, 2016). To this point, replications and null reporting are often overlooked for publication because cultural incentives emphasize novelty over verification (Nosek et al., 2012), indicating a disconnect between the push for better science and the academic publishing community. This is important for policy makers to know because transparency in influenza research can save lives (Besançon et al., 2021). To learn more about open science in the virology community, the Open Scholarship Survey asked influenza researchers about their beliefs, behaviors, and perceptions regarding open science practices for replication and null results reporting.

**METHODS**

The Open Scholarship Survey (77 questions total) was distributed by the Center for Open Science from February to March, 2021. Data were collected from 228 researchers within the fields of influenza and virology research, globally. This initiative was funded by Flu Lab.

**RESULTS**

Overall, the influenza community reported favorable attitudes toward the open science practices of replication and null results reporting. However, these same respondents perceived their peers to have much less favorable views toward these same activities (see Figure). A minimal number of respondents indicated that they included a replication (22%) or null result (15%) in their most recent publication.

**CONCLUSION**

The presented data demonstrate a disconnect between what influenza researchers believe regarding open science and how influenza researchers think their peers see open science practices. This disconnect may partially explain why influenza researchers report favorable views toward open science practices, such as replication and null results reporting, but do not engage in these practices regularly. Illuminating the overall favorability of influenza researchers toward open science practices may ultimately lead to a more transparent influenza research process. This is important for policy makers to focus on because transparency in influenza research can save lives (Besançon et al., 2021).
**Effectiveness of adjuvanted trivalent vaccine (aTIV) for influenza over 18 epidemic seasons**

**Topic:** Why influenza and RSV disease are a priority for policy makers

Francesco Lapi (1), Alessandro Rossi (2), Aurelio Sessa (2), Claudio Cricelli (2)

1: Health Search, Italian College of General Practitioners and Primary Care, Italy; 2: Italian College of General Practitioners and Primary Care

**BACKGROUND**

Various studies support the higher effectiveness of MF59*-adjuvanted trivalent vaccine (aTIV) compared to non-adjuvanted trivalent (TIV) or quadrivalent (QIV) influenza vaccine in preventing influenza-related hospitalizations in the elderly. The aims of this study are to evaluate the relative effectiveness of aTIV compared to TIV/QIV in preventing all-cause hospitalization or hospitalization for respiratory causes across 18 seasons.

**METHODS**

A nested case-control analysis of older adults (> 65 years) was conducted from 2001/2002 to 2018/2019 influenza seasons in Italy, using the Health Search Database (HSD), an Italian data source of primary care. The odds ratio (OR) with 95% confidence intervals (CI) of the outcomes in elderly patients being vaccinated with MF59-TIV or TIV/QIV was estimated by conditional logistic regression.

**RESULTS**

In a cohort of 58,252 older individuals included, the use of aTIV compared to TIV/QIV was associated to a 12% significantly lower risk of hospitalization (OR=0.88; 95% CI: 0.80-0.98). When the outcome was limited to hospitalizations due to respiratory causes, the use of aTIV was associated with a 37% lower risk than TIV/QIV (OR=0.63; 95% CI: 0.44-0.91).

**CONCLUSIONS**

Across 18 influenza seasons, aTIV was related to a reduction of the risk of all-cause or respiratory-related hospitalizations events, when compared to nonadjuvanted TIV/QIV in older individuals. These findings support the public health providers in prevention strategies of influenza.
The Economic Burden of Influenza Among Adults Aged 18-64: A Systematic Literature Review

**Topic:** Societal impact of influenza, RSV disease and COVID-19

Caroline de Courville (1), Sarah M Cadarette (2), Erika Wissinger (2), Fabián P Alvarez (1)

1: Sanofi Pasteur, France; 2: Xcenda, L.L.C., Carrollton, TX

**BACKGROUND**

While the economic burden of influenza infection is well described among adults aged 65 and older, less is known for other adult age groups. A systematic literature review was conducted to describe the economic burden of seasonal influenza in adults aged 18-64 year-old (yo), to identify the main determinants of direct and indirect costs and to highlight any gaps in the existing published evidence. A stratification of the data by age group (18-49yo / 50-64yo) and at-risk status was completed to assess whether specific groups are associated with a higher economic burden.

**METHODS**

Peer-reviewed publications were searched in Medline and Embase databases from 2007, and conference proceedings from 2018, both up to the search date (February 7, 2020). In addition, bibliographies of SLRs and meta-analyses captured by the search were reviewed to identify any relevant missing publications. Publications were then screened against the inclusion and exclusion criteria and resulting trials, real-world observational studies, or cost-effectiveness studies were included only if primary influenza-related cost data (direct or indirect) or absenteeism data were reported.

**RESULTS**

Of the 2,613 publications screened, 51 studies were included in this review. Half of the included studies were conducted in the United States (US), and most of them (88%) described patients with influenza-like illness rather than laboratory-confirmed disease. Only 12 studies reported cost data specifically for an at-risk population. The most frequently reported outcomes were hospitalization costs, overall direct costs and number of workdays lost.

Overall, the extracted data highlighted that within the 18-64 yo group, most of the economic burden of influenza was attributable to indirect costs (up to 88%), whereas up to 79% of overall direct costs were attributable to hospitalizations. Furthermore, within the 18-64 yo group influenza-related costs increased with age and underlying medical conditions. The reported cost of influenza-related hospitalizations was found to be up to 2.5 times higher among at-risk compared to not-at-risk populations.

**CONCLUSIONS**

This review documents the considerable economic impact of influenza among adults aged 18-64. In this age group, most of the influenza costs are indirect. These costs are generally not recognised by decision makers, especially in economic evaluations, leading to an underestimation of the economic impact of influenza. Furthermore, this review highlighted significant gaps in the literature, limiting generalizability and interpretation. In order to address current gaps, more studies are needed with a focus on the economic burden of influenza outside the US, on those at-risk of severe outcomes and on lab-confirmed influenza patients.

This work was funded by Sanofi Pasteur
Evaluation of Psychometric Properties of a Patient-Reported Outcome Measure for the Assessment of COVID-19 Signs and Symptoms: A Cross-Sectional Observational Study

Topic: Societal impact of influenza, RSV disease and COVID-19

Valene Williams (1), Carla DeNute Ramana (1), Shen Fehnel (1), Jeffery Stoddard (2), Jerald Sadoff (3), Sandy Lewis (1), Stuart Yarr (1), Jia Ma (1), Yan Liu (2), Eva G. Katz (2), Kelly McQuarrie (4), Chenglong Han (2), Ashley F. Slagle (5), Eric K. H. Chan (2)

1: RTI Health Solutions, Research Triangle Park, NC, USA; 2: Janssen Global Services LLC, Raritan, NJ, United States of America; 3: Janssen Research and Development, Raritan, NJ, USA; 4: Janssen Global Services LLC, Horsham, PA, USA; 5: Aspen Consulting LLC, Steamboat Springs, CO, USA

BACKGROUND
We conducted a cross-sectional, observational study in the United States to evaluate preliminary psychometric properties of a patient-reported outcome (PRO) measure, the Symptoms of Infection with Coronavirus-19 (SIC).

METHODS
Participants aged ≥18 years with a self-reported positive COVID-19 test within the previous 2 weeks and ≥2 COVID-19 symptoms completed the web-based SIC, which assesses the presence of 30 signs/symptoms of COVID-19, and the severity of 25, if present. To evaluate the SIC, participants completed additional PRO measures, including PGIS, PGIC, PROMIS-29, EQ-5D-5L, and a symptom checklist. Inter-item correlations and Cronbach’s alphas were calculated to evaluate the SIC structure, scoring, and reliability. Correlations were computed to evaluate the construct validity of the SIC items and composite scores. Known-group analyses evaluated the discriminating ability of the SIC.

RESULTS
152 participants completed the PRO measures (mean ± SD age: 51 ± 18.6 y, female: 62%). Most participants reported moderate symptoms (47%) versus mild (26%) or severe (26%) for a mean duration of 14.9 ± 7.3 days. The most frequently endorsed SIC symptoms were fatigue (78%), feeling unwell (66%), cough (61%), physical weakness (60%), and headache (60%). Fatigue had the highest severity rating (mean=4.58; scale 0–10 [ “none” − “worst possible” ] in those <65 y, followed by headache (3.78), feeling unwell (3.43), and physical weakness (3.36); in those ≥65 y, fatigue (4.80) was followed by feeling unwell (4.23), loss of appetite (3.61), and physical weakness (3.44). All SIC inter-item correlations were positive; within each item set describing different bodily systems, correlations were mostly moderate (r≥0.3), statistically significant, and supporting the creation of SIC composite scores. The internal consistency reliabilities of SIC composite scores were satisfactory (Cronbach’s alpha range, 0.69–0.91). Construct validity correlations between SIC items and PROMIS-29 scores were positive or negative as hypothesized, e.g., the SIC fatigue item correlated positively with the PROMIS-29 fatigue score (r=0.64) and negatively with the physical function score (r=-0.65). PROMIS-29 physical function correlated moderately/strongly with SIC physical weakness (r=−0.67), muscle aches/pains (r=-0.52), and joint aches/pains (r=-0.42). SIC composite scores correlated moderately/strongly with PROMIS-29 scores; all were statistically significant. Most EQ-5D-5L scores correlated moderately/strongly with SIC composite scores; pain/discomfort, self-care, and usual activities correlated most strongly with SIC constitutional and musculoskeletal scores (r range, 0.58–0.65). In the known-groups analyses, all item-level group differences were in the correct direction, and most were statistically significant; all composite-level differences were in the correct direction and statistically significant. The no-symptoms/mild PGIS subgroup achieved better SIC scores versus the moderate/severe PGIS subgroup.

CONCLUSIONS
The results support the reliability and validity of the SIC items and composite scores as appropriate and useful measures of COVID-19 symptom severity for use in clinical trials of COVID-19 vaccines and treatments in adults.

Acknowledgement/Funding Statement Supported by Janssen Vaccines & Prevention B.V.
Qualitative Interviews from a Cross-Sectional Study to Support the Content Validity of a Patient-Reported Outcome Measure of COVID-19 Signs and Symptoms in Adults

**Topic:** Societal impact of influenza, RSV disease and COVID-19

**Carla DeMuro Romano (1), Margaret Mayorga (1), Sheri Fehnel (1), Jeffrey Stoddard (2), Jerald Sadoff (3), Sandy Lewis (1),iva G. Katz (2), Kelly McQuarrie (4), Ashley F. Slagle (5), Eric K.H. Chan (2)**

1: RTI Health Solutions, Research Triangle Park, NC, United States of America;  
2: Janssen Global Services LLC, Raritan, NJ, USA;  
3: Janssen Research and Development, Raritan NJ, USA;  
4: Janssen Global Services LLC, Horsham, PA, USA;  
5: Aspen Consulting LLC, Steamboat Springs, CO, USA

**BACKGROUND**

The Symptoms of Infection with Coronavirus-19 (SIC) is a 30-item patient-reported outcome (PRO) measure designed to assess the presence and severity of signs/symptoms of COVID-19. A cross-sectional study was conducted for preliminary psychometric evaluation of the SIC. As part of this study, an exit survey was administered and qualitative interviews conducted (in a subset of participants) to collect additional evidence to support the content validity of the SIC.

**METHODS**

A cross-sectional observational study was conducted in adults ≥ 18 years of age in the United States who self-reported ≥ 2 COVID-19 symptoms and a positive COVID-19 test within 2 weeks of screening. Participants completed a set of web-based PRO measures. Two additional questions asking participants to identify symptoms needing to resolve to return to usual activities and 4 questions about access/barriers to COVID-19 testing and care were also included. From study participants who agreed to a follow-up qualitative telephone interview, a subset was selected to represent a diverse population, targeting ≥ 50% minority representation.

**RESULTS**

A total of 152 participants completed the survey and 20 completed follow-up interviews. Participants who completed the follow-up interview were racially diverse (African American, 45% [9/20]; white, 25% [5/20]; American Indian, 5% [1/20]; mixed race, 10% [2/20]; other, 15% [3/20]). A small majority were women (65% [13/20]) and the mean age was 51.5 y. Almost half (45% [9/20]) had a preexisting risk factor increasing the likelihood of severe COVID-19 outcomes, and 45% (9/20) were hospitalized due to COVID-19. Participants described a wide variety of signs and symptoms, the most common being fatigue, cough, shortness of breath, and decreased sense of smell and taste. Shortness of breath, headache, and fatigue were identified as the greatest barriers to return to usual activities. Consistent with prior qualitative research in this population, participants could describe differences in COVID-19-related symptoms as compared to symptoms common to other health conditions or associated with their baseline health/underlying comorbidities. Participants reported hesitancy to self-identify their COVID-19 experience as severe disease if not hospitalized or requiring supplemental oxygen, despite half (9/20; 45%) reporting symptoms consistent with severe disease. Most participants (17/20; 85%) reported that their lives were impacted by needing to self-isolate and experiencing anxiety regarding what would happen to them/family members. Interview participants endorsed the content and format of the SIC and described the measure as straightforward, comprehensive, and easy to self-complete, even when ill. Overall, participants felt the SIC was an accurate measure to report and track COVID-19 symptom onset, emergence of new signs/symptoms, and changes in symptoms over time.

**CONCLUSIONS**

Results of the qualitative interviews in this more diverse and vulnerable population were consistent with previous qualitative research in a predominately white sample, further supporting the content validity and format of the SIC.

**Acknowledgement/Funding Statement:** Supported by Janssen Vaccines & Prevention B.V.
Influenza vaccination coverage rates during the COVID-19 pandemic: data from seven countries in the Northern Hemisphere

**Topic:** Benefits of vaccinating healthcare workers and other risk groups

Marco Del Riccio (1,2), Sytske Wiggersma (1), Lisa Staadegaard (1), Murat Akçay (3), Clotilde El Guerci-Sébèlaine (3), Saverio Caimi (1), John Paget (1)

1: Netherlands Institute for Health Services Research, Italy;
2: Postgraduate Medical School in Public Health, University of Florence, Italy;
3: Sanofi Pasteur: Medical Evidence generation, Lyon, France

**Background**

The World Health Organization (WHO) recommends the annual vaccination of elderly individuals (aged 65+) to prevent influenza infections, reduce the severity of disease and complications or deaths. In addition, WHO SAGE has defined this group as one of the highest priority groups to receive the influenza vaccine during the COVID-19 pandemic.

Within the FluCov project (www.nivel.nl/en/fluco), which aims to better understand the impact of SARS-CoV-2 on influenza activity in 21 countries worldwide, data on influenza vaccination coverage rates (VCRs) in the elderly were collected. Here we report the preliminary results of the VCR data collection and their evolution.

**Methods**

Reports and websites of Public Health Institutes and International Organizations were reviewed from 2019 to 2021 to collect influenza VCRs in the elderly in the pandemic and pre-pandemic periods; along with specific information on the target population. VCR data were extracted and the difference between 2019/20 and 2020/21 calculated.

**Results**

As of August 11th, data on influenza VCRs in the elderly for both the 2020/21 and 2019/20 winter seasons were collected for seven countries: France, Italy, Israel, South Korea, UK, and USA (Table 1). We were unable to find updated VCR data for 14 countries. During the winter of 2020/21, influenza VCRs in the elderly increased in France (+7.9%), Italy (+10.7%), Spain (+13.6%, provisional data), Israel (+8.4%), UK (+8.5%), and USA (+10.3% provisional data). South Korea was the only country where a decrease was observed (-10.2%).

**Conclusions**

In most of the countries for which data were available, we observed an unprecedented increase in the demand for influenza vaccine in the elderly; remarkably, the USA and the UK have managed to reach coverage rates of 75% which is recommended by WHO. The low levels of influenza activity since the start of the COVID-19 pandemic and the small number of cases observed during the 2020/21 winter may lead to an increase of the susceptible population. It is therefore important to maintain high VCRs to better prevent a potential resurgence of influenza cases during the coming winter.

[1] Australia, Brazil, Canada, China, Germany, India, Japan, Mexico, Philippines, Poland, Thailand, The Netherlands, Taiwan, Vietnam.

<table>
<thead>
<tr>
<th>Country</th>
<th>Age</th>
<th>2019/20 VCR (%)</th>
<th>2020/21 VCR (%)</th>
<th>VCR difference (%)</th>
<th>Source</th>
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</thead>
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<tr>
<td>France</td>
<td>65+</td>
<td>52.0</td>
<td>59.0</td>
<td>+ 7.9</td>
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</tr>
<tr>
<td>Israel</td>
<td>65+</td>
<td>60.0</td>
<td>68.2</td>
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<td>76.3</td>
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<td>South Korea</td>
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<td>73.6</td>
<td>- 10.2</td>
<td>Kwon Y. et al., 2021, Vaccines</td>
</tr>
<tr>
<td>Spain</td>
<td>65+</td>
<td>54.6</td>
<td>66.2 (provisional)</td>
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<td>40.1 (provisional)</td>
<td>- 10.3</td>
<td>Centers for Disease Control and Prevention (CDC)</td>
</tr>
</tbody>
</table>

Table 1: Influenza VCRs
Attitude and beliefs about the seasonal influenza vaccination among Health Care Workers during Covid-19 Pandemic - Kashmir, India

Topic: Benefits of vaccinating healthcare workers and other risk groups

Hyder Manzoor (1), Faisal Irshad (2), Faheem U din (1), Rafi Jan (1), Parvaiz Koul (1)

1: Sheri kashmir institute of medical sciences, India;
2: Indian Institute of Integrative Medicine, Jammu, India

BACKGROUND

The 2019/20 influenza season in northern hemisphere coincided with the first wave of Covid-19 pandemic. We set out to assess the uptake of influenza vaccine during Covid-19 pandemic, the reasons for refusing vaccination in HCWs and to identify potential modifiers of the decision-making process regarding the uptake of the 2020/21 influenza vaccine.

METHODS

A cross sectional study was conducted at Sheri kashmir Institute of Medical Sciences from March 2020 to June 2020, a period which witnessed the country wide lockdown in India due to the ongoing coronavirus pandemic. Of the 494 participants in the study, 277 (56%) 83 belonged to physician/physician trainees group, 39 to nurse/nurse trainees group, 82 to allied health professionals group, 24 to support staff group, 05 to administrative department and 44 to others*) had received the flu vaccine, even as 87% (n=430) considered influenza as a potentially severe disease and 95.7% (n=508) were aware about the existence of vaccine against influenza. Ineffectiveness of the influenza vaccine (14%) was the main reason cited by the participants for not getting vaccinated. Even though 84% (416) of the HCWs agreed that vaccines are generally beneficial, 35.4% believed that adverse effects of the vaccines are under reported. 77.3% (382) participants intended to get vaccinated for the next influenza season. Vaccine uptake recorded a 12 times increase since the previous KAP study conducted in 2012. One time flu shot for life long immunity, raising awareness, free of cost availability and effectiveness of vaccine were the suggestions put forth by HCWs in order to increase the vaccine uptake.

RESULTS

Of the 494 participants in the study, 277 (56%) 83 belonged to physician/physician trainees group, 39 to nurse/nurse trainees group, 82 to allied health professionals group, 24 to support staff group, 05 to administrative department and 44 to others*) had received the flu vaccine, even as 87% (n=430) considered influenza as a potentially severe disease and 95.7% (n=508) were aware about the existence of vaccine against influenza. Ineffectiveness of the influenza vaccine (14%) was the main reason cited by the participants for not getting vaccinated. Even though 84% (416) of the HCWs agreed that vaccines are generally beneficial, 35.4% believed that adverse effects of the vaccines are under reported. 77.3% (382) participants intended to get vaccinated for the next influenza season. Vaccine uptake recorded a 12 times increase since the previous KAP study conducted in 2012. One time flu shot for life long immunity, raising awareness, free of cost availability and effectiveness of vaccine were the suggestions put forth by HCWs in order to increase the vaccine uptake.

CONCLUSION

Our data suggests that the uptake of vaccine enhanced significantly during the past decade with the highest increase recorded when the vaccines were provided free of charge. Creating awareness regarding IV among HCWs is of utmost importance in order to eliminate the fear of vaccine related severe adverse effects and misconceptions regarding its effectiveness. In light of the COVID-19 pandemic, identifying the hurdles to IV uptake among health care workers may also assist in developing successful awareness strategies to promote the Covid19 vaccination.

*Others group are the employees belonging to Class IV which includes Peon, sweepers, sanitary staff etc.

#ESWI2021 Creating awareness regarding IV among HCWs is of utmost importance in order to eliminate the fear of vaccine related severe adverse effects and misconceptions regarding its effectiveness. In light of the COVID-19 pandemic, identifying the hurdles to IV uptake among health care workers may also assist in developing successful awareness strategies to promote the Covid19 vaccination.
Cost-Effectiveness of High-Dose Quadrivalent Influenza Vaccine (HD-QIV) Versus Adjuvanted Quadrivalent Influenza Vaccine (aQIV) in the Italian elderly population

**Topic:** Benefits of vaccinating healthcare workers and other risk groups

Filippo Rumi (1), Michele Basile (1), Americo Cicchetti (1), Fabian Alvarez (2), Barbara Muzii (3), Maria Vittoria Azzi (3)

1: Alta Scuola di Economia e Management dei Sistemi Sanitari, Università Cattolica del Sacro Cuore, Roma, Italy; 2: Sanofi Pasteur Health Economics and Value Assessment, Lyon, France; 3: Sanofi Pasteur Market Access, Milan, Italy

**BACKGROUND**

Influenza is a widespread acute respiratory disease that represents a serious public health problem, both from the National Health System (NHS) in Italy and societal perspectives. The High Dose quadrivalent influenza vaccine (HD-QIV), containing 4 times the amount of antigens of the Standard Dose (SD-QIV) vaccine, is the only influenza vaccine to have demonstrated higher clinical efficacy in randomized clinical trial than the SD-QIV in protection from lab-confirmed influenza (rVE = 24.2%) and reduction in hospitalizations for cardiorespiratory events (+18.2%) in the population 65 years and above. The objective of this study was to estimate the cost-effectiveness of HD-QIV compared to the standard of care for the Italian elderly population, the adjuvanted quadrivalent vaccine (aQIV).

**METHODS**

The analysis has been conducted from the perspective of the NHS and it is based on a decision tree model comparing the vaccination with HD-QIV vs. aQIV within a 1-year time-horizon. The model estimates the health outcomes for both vaccines: GP visits, ED visits, hospitalizations, deaths, life years (LYs) and quality-adjusted life-year (QALYs). In particular, two different hospitalizations approaches are considered: hospitalization conditional on developing influenza and hospitalization possibly related to influenza. In the first case, only hospitalizations with influenza diagnosis ICD-10-CM codes are considered. The second approach includes hospitalizations for cardiorespiratory events possibly related to influenza, thus allowing to consider the effect of HD-QIV in reducing these influenza-related complications.

Considering that there is no RCT documenting superior efficacy of aQIV vs SD-QIV, the analysis assumes relative equal efficacies (rVE) for aQIV and SD-QIV for both, influenza cases and cardiorespiratory events. In order to account for uncertainty on this parameter, alternative scenarios with rVE of aQIV vs SD-QIV=6% and 12% were considered.

**RESULTS**

Considering hospitalizations directly attributable to influenza, the results highlighted that the vaccination strategy with HD-QIV is cost-effective in all the scenarios described (rVE for aQIV vs SD-QIV equal to 0%, 6% and 12%) with an incremental cost-effectiveness ratio (ICER) of 7,301€, 9,805€ and 14,733€ per QALY, respectively.

When considering hospitalizations possibly related to influenza, a dominant cost-effectiveness profile emerges in the comparison with aQIV. Considering only hospitalization cost, the use of HD-QIV implies savings of -176 million €. Deterministic and probabilistic sensitivity analysis confirm the robustness of the results reported.

**CONCLUSIONS**

Vaccination with HD-QIV instead of aQIV among Italian elderly could annually reduce the public health burden of influenza and its related complications. The results highlight that the vaccination strategy with HD-QIV would be cost-effective if only hospitalizations conditional on developing influenza are included; and cost-saving when the full burden of influenza is considered.

**Keywords:** Economic Evaluation, Cost-Effectiveness Analysis. Influenza, Vaccines

**Disclosures** Financial support: This study was funded by Sanofi.
**Ad26.RSV.pref-based vaccine regimen induced antibody Fc-effector functions and neutralization are associated with protection from respiratory syncytial virus infection**

*Topic: Innate and adaptive immunity towards influenza, RSV disease and COVID-19*

Yannic C. Bartsch (1), Deniz Cizmeci (1, 2), Jaewon Kang (1), Nickita Mehta (1), Tomer Zohar (1, 2), Sivakumar Periasamy (3), Jeroen Tolboom (4), Jerry Sadoff (4), Christy Comeaux (4), Benoit Callendret (4), Nicolas Noulin (5), Alexander Bukreyev (3), Douglas A. Lauffenburger (2), Arrangassery Rosemary Bastian (4), Galit Alter (1)

1: Ragon Institute of MGH, MIT, and Harvard, Cambridge, MA United States of America; 2: Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA USA; 3: Department of Pathology, University of Texas Medical Branch, Galveston, TX USA; 4: Janssen Vaccines & Prevention BV, Leiden, The Netherlands; 5: hVIVO Services Limited, London, England UK

**BACKGROUND**

Respiratory Syncytial Virus (RSV) infection is a major cause of severe lower respiratory tract infection and death in young infants and the elderly. With no licensed prophylactic treatment available, current investigational vaccine candidates aim to elicit neutralizing antibody titers. However, neutralizing and binding antibody titers have poorly predicted protection against natural infections in the past, and accumulating data across epidemiologic cohorts and animal model studies collectively point to a role for additional antibody Fc-effector functions in protection against RSV. However, whether vaccine-induced neutralizing antibodies, Fc-effector functions, or a combination of antibody mechanisms are linked to protective immunity remains unclear but could provide key insights for the design of next generation protective RSV vaccines.

**METHODS**

To begin to define the humoral correlates of immunity against RSV, here we profiled an Ad26-RSV.pref vaccine which induced RSV-specific humoral immune response in a group of healthy adults that were ultimately challenged with RSV.

**RESULTS**

Vaccination induced robust F-specific humoral immune responses, marked by a strong induction of pref-specific antibody titers, neutralization, and Fc-effector functions. Protection from infection was clearly linked to opsonophagocytic functions, driven by mucosal relevant IgA and differentially glycosylated RSV-specific IgG profiles, marking a functional mucosal humoral immune signature of protection against RSV. Further, the addition of recombinant RSV pref protein to Ad26-RSV.pref increased the relevant antibody functions that were shown to be correlated with protection.

**CONCLUSION**

Our data suggest a critical role of Fc-mediated effector functions beyond neutralization for the prevention of RSV infection which was induced by Ad26-RSV.pref.
Relative Vaccine Effectiveness of Cell-Based Quadrivalent Influenza Vaccine in Three Consecutive Influenza Seasons in the United States

**Topic:** Strategies for future Influenza vaccination

Constantina Boikos (1), Ian McIveren (2), Deborah Malrine (2), Justin K. Orthz (3), Jaan Puig-Barbara (4), Chris Clarke (5), Mendel Haag (6)

1: Seqirus CA Inc., Canada; 2: Seqirus USA Inc.; 3: University of Maryland School of Medicine, USA; 4: FISABIO, Valencia, ESP; 5: Seqirus AUS Inc.; 6: Seqirus NLD Inc.

**BACKGROUND**

The cell-based quadrivalent inactivated influenza vaccine (IIV4c) contains viruses grown in mammalian cell lines rather than eggs. IIV4c is therefore not subject to egg-adaptive changes which can result in antigenic mismatch and which may contribute to reduced vaccine effectiveness. As a consequence, IIV4c is expected to offer improved protection relative to egg-based vaccines, although this benefit needs to be evaluated across multiple influenza seasons as the impact of egg adaptation can vary between seasons. Therefore, the relative vaccine effectiveness (rVE) of IIV4c versus egg-derived quadrivalent influenza vaccines (IIV4e) in preventing influenza-related medical encounters (IRMEs) in children and adults was estimated for 3 consecutive U.S. influenza seasons with different circulating strains, of which the impact of egg adaptation was most pronounced in 2017/18.

**METHODS**

Three retrospective, observational cohort studies conducted during the 2017-2018, 2018-2019 and 2019-2020 U.S. influenza seasons included 18.4 million immunizations (IIV4c or IIV4e) in persons aged ≥4 years. The data source combined electronic medical records with pharmacy and medical claims data if available. The outcome was influenza-related medical encounters (IRME) identified from patient records using codes specific to influenza disease diagnosis (ICD J09*–J11*). Vaccine effectiveness was estimated using propensity score methods adjusting for prespecified confounders (age, sex, race, ethnicity, geographic location, week of vaccination, health status). Subgroup analyses included specific age groups (pediatric 4-17, adult 18-64 and older adult ≥65 years) and those with high-risk medical conditions.

**RESULTS**

IIV4c demonstrated a consistent benefit over IIV4e in the prevention of IRMEs in the overall population and in pediatric and adult subgroups over the three consecutive U.S. influenza seasons, except for the 4-17 year age group in 2017-2018 and adults ≥65 years in all seasons (Figure). The rVE for the overall population ranged from 7.8% for 2018-2019 season to 19.2% for 2017-2018 season.

**CONCLUSIONS**

IIV4c has consistently demonstrated better relative effectiveness than IIV4e across all seasons assessed to date which were characterized by different dominant circulating strains, and different levels of drift or egg adaptation.
Immunogenicity and efficacy of different heterologous prime-boost vaccination regimens against swine H1N1 influenza viruses

*Topic: Strategies for future Influenza vaccination*

Anna Parys (1), Elien Vandoorn (1), Katharina Passvogel (2), Walter Fuchs (2), Thomas C. Mettenleiter (2), Kristien Van Reeth (1)

1: Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke, Belgium; 2: Institute of Molecular Virology and Cell Biology, Friedrich Loeffler Institute, Federal Research Institute for Animal Health, 17493 Greifswald Insel Riems, Germany

In previous heterologous prime-boost vaccination studies in pigs, we used whole inactivated virus vaccines (WIV) based on antigenically different strains within the H3N2 or H1N1 subtype. This strategy induced broader antibody responses and superior protection as compared to priming and boosting with homologous vaccine. However, we failed to obtain pan-H1N1 or pan-H3N2 neutralising antibody responses. Here, we combined different vaccine strains, vaccine platforms and routes of vaccination to stimulate both local and systemic immune responses and further broaden immune responses and protection.

Pigs were primed with a vaccine based on the 2009 pandemic H1N1 virus (pdm09), either by intranasal administration of a live attenuated Suid herpesvirus 1 vector expressing the pdm09 hemagglutinin (vector-pdm09, 3 groups) or by intramuscular injection of WIV (WIV-pdm09, 3 groups). Four weeks later, WIV based on 3 different H1N1 strains (homologous pdm09, and 2 heterologous H1N1 swine influenza A virus (swIAV) strains) were used for the booster vaccination of vector- and WIV-primed groups. Two control groups received a mock vaccination or a single vaccination with vector-pdm09. Following vaccination, we examined antibody-secreting cell (ASC) responses in peripheral blood, draining lymph nodes, and nasal mucosa (NMC) by ELISPOT assays. Six weeks after the second vaccination, pigs were challenged intranasally with pdm09 or 1 of 2 heterologous H1N2 swIAV. At this timepoint, serum was collected for evaluation of hemagglutination inhibition (HI) antibody titers against a panel of 24 antigenically diverse H1 viruses. Three days later, pigs were euthanized to collect respiratory tract samples for virus titration.

Two weeks after the first, intranasal vaccination with vector-pdm09, we observed a strong IgA ASC response in the NMC. Following the booster vaccination with heterologous WIV, IgA ASC in NMC were boosted. Additionally, numbers of IgG secreting PBMC were up to 4-fold higher in the vector-pdm09 / heterologous WIV groups than in the WIV-pdm09 / heterologous WIV groups. On the other hand, IgG ASC responses in the draining lymph nodes were up to 12-fold higher in the latter. The breadth of the serum HI antibody response was similar with all 3 heterologous prime-boost regimens examined. That is, we detected seroprotective HI titers (≥40) against 29-38% of H1 viruses in vector-pdm09 / heterologous WIV groups. 21-33% in WIV-pdm09 / heterologous WIV groups, and 29% in vector-pdm09 WIV-pdm09 group. A single vector-pdm09 administration resulted in sterile protection against homologous challenge. However, no vaccination regimen could induce sterile protection against heterologous challenge and we found no differences in protection between vaccination regimens.

Although the intranasal vector-pdm09 vaccination followed by a heterologous intramuscular WIV vaccination did induce a strong IgA ASC response in the NMC, this was not associated with a broader serum HI antibody response or superior protection compared to WIV-pdm09 – heterologous WIV vaccination.
Lower infectious viral load in respiratory samples of vaccinated compared to unvaccinated COVID-19 patients

**Topic:** Viral and host factors in the transmission of influenza, RSV disease and COVID-19

Benjamin Meyer (1), Olha Puhach (2), Pauline Vetter (3,4), Kenneth Adea (2), Pascale Sallonneau (2), Frédérique Jacquieraz Bausch (3,5,6), Laurent Kaiser (3,4), Isabella Eckerle (2,3,4)

1: University of Geneva, Switzerland; 2: Department of Microbiology and Molecular Medicine, University of Geneva, Geneva, Switzerland; 3: Geneva Centre for Emerging Viral Diseases, Geneva University Hospitals, 1205 Geneva, Switzerland; 4: Laboratory of Virology, Division of Laboratory Medicine, Geneva University Hospitals & Faculty of Medicine, University of Geneva, 1205 Geneva, Switzerland; 5: Division of Tropical and Humanitarian Medicine, Geneva University Hospitals, Geneva, Switzerland; 6: Primary Care Division, Geneva University Hospitals, Geneva, Switzerland

There is an increasing number of breakthrough infections in COVID-19 vaccinated individuals. Onward transmission from infected patients, that are fully vaccinated, has been reported especially in the context of the SARS-CoV-2 Delta variant. However, it remains unclear to what extent this occurs and if vaccinated patients shed the same amount of infectious virus compared to unvaccinated patients. Viral genome copies were found to be similar in respiratory specimens of vaccinated and unvaccinated patients. However, viral genome copies are often poorly correlated with infectious virus shedding.

In this study we quantified the infectious viral load in nasopharyngeal swabs of patients infected with the original SARS-CoV-2 strain (n=118) as well as vaccinated (n=132) and unvaccinated (n=83) individuals infected with the Delta variant using a focus forming assay. In addition, we determined infectiousness of samples by virus isolation success and compared the results to the number of genome copies. Patients were matched in regard to sex, age and days post symptom onset to eliminate possible confounders. As expected, we observed a poor correlation between the number of viral genome copies and infectious viral titers, whereas virus isolation success was largely concurrent with viral titers. We found elevated infectious viral titers in unvaccinated patients infected with the Delta variant compared to those infected with original SARS-CoV-2. This difference was especially marked 3 days post symptom onset. Next, we compared infectious viral titers in vaccinated and unvaccinated matched patients infected with the Delta variant. Interestingly, vaccination reduced the infectious viral load in patients approximately 10-fold starting already at one day post symptom onset.

In conclusion, we could show that vaccination led to a significant reduction and faster clearance of infectious virus particles, thereby potentially limiting transmissibility. Furthermore, we found that infection with the Delta variant led to the elevated and prolonged infectious virus shedding compared to the original virus strain contributing to the observed higher transmissibility of the Delta variant.
The regulatory role of NS gene mRNA secondary structure in influenza virus life cycle

**Topic:** Virus structure and replication in influenza virus, RSV and SARS-CoV-2; latest developments in influenza virus, SARS-CoV-2 and RSV molecular virology

**Irina Baranovskaya (1,2), Mariia Sergeeva (2), Alexey Lozhkov (1,2), Aleksandr Taraskin (1,2), Andrey Vasin (1,2)**

1: Peter the Great St. Petersburg Polytechnic University, Russian Federation; 2: Smorodintsev Research Institute of Influenza, Russian Ministry of Health, Russian Federation

**BACKGROUND**

Currently, understanding of the functions of influenza structural RNA elements is rather limited. The formation of stable NS mRNA secondary structures has been demonstrated earlier for two regions (nucleotide positions 82-148 and 497-564) near 5' and 3' splice sites. The NS gene encodes nonstructural protein NS1 and nuclear export protein NEP. It has been predicted that the type of these structures may vary between influenza A strains, including the pandemic virus A/Brevig Mission/1/1918 (H1N1) and highly virulent bird-adapted H5N1 strains. The purpose of our study is to elucidate the role of the NS mRNA secondary structures in influenza A reproduction in vitro and in vivo.

**METHODS**

Four viral variants, with different combinations of secondary structure in two NS mRNA regions (82-148 and 497-564), were obtained in these studies by reverse genetics based on the laboratory strain A/Puerto Rico/8/1934 (H1N1). MDCK (RR #F-58), A549 cells (ATCC CCL-185) and the Vero cell line (ATCC, #CCL-81) were used to perform in vitro research. Female BALB/c mice (18–20 g, aged 6-8 weeks) were purchased from the Stoibovaya Biomedical Scientific Center FMBA (Russia). Infectious activity of virus samples was measured by titration in cells (the 50% Tissue Culture Infectious Dose determination). For quantitative measurement of viral proteins, ELISA and western blotting were used. All studies were approved by the local ethics committee of Smorodintsev Research Institute of Influenza.

**RESULTS**

NS mRNA secondary structures were found to affect the production of viral proteins in infected cells: a hairpin in the region (82–148) enhances NS1 production; a hairpin in the region (497–564) in the absence of a stable structure in the region (82–148) reduces the effective translation of NEP. The study of replication of the obtained influenza viruses in sensitive cells (A549, Vero, MDCK) did not show any differences between the strains during multi-cycle infection and in the early stages of infection. Differences in the viral pathogenicity to mice were demonstrated. It was found that the virus characterized by the absence of an RNA hairpin in the region (82–148) and a stable hairpin in the region (497–564) of the NS mRNA is less pathogenic in laboratory animals compared to other viruses, according to determination of viral infectious activity in the lungs.

**CONCLUSIONS**

A specific combination of RNA secondary structures of the NS gene (presence of a hairpin at position 497-564 and absence of a hairpin structure at position 82-148) leads to decreased NS1 and NEP expression in infected cells and causes the reduced infectious activity of the virus in infected animal lung.

*This work was supported by Russian Science Foundation grant 18-7400130.*
Poster abstracts
Development and characterization of an aerodynamic system for pulmonary delivery of influenza vaccine

**Topic:** Antiviral and immune therapy for influenza, RSV disease and COVID-19

**Saurabh Bhargava (1), V Bhargava (2)**

1. United Institute of Pharmacy, India;  
2. GTB Hospital, India

The name *influenza* is Italian and means “influence”. Commonly referred to as the flu, it is an infectious disease caused by RNA viruses of the family Orthomyxoviridae, that affects birds and mammals. The aim is also to develop and characterize aerodynamic systems with r-H1N1AgS for safely deposition in alveoli to enhance the bioavailability and control release of influenza antigen after pulmonary administration in animal model. This Induces not only systemic humoral (IgG) responses, but also cell-mediated (IL-4, IFN-γ) and mucosal immune responses (IgA, IgG), non-invasive, propellant & needle free delivery of vaccine.

The chitosan microparticles were prepared by ionic gelation method of chitosan with tripolyphosphate(TPP). The formulations were optimized on the basis of particle size, tap density & entrapment efficiency. The external morphology of the optimized formulation was studied by TEM & SEM. The zeta potential was determined along with stability studies at accelerated temperatures. The in-vivo studies involved determination of antibody titres in serum and mucosal secretions and uptake studies by fluorescence microscopy.

The results show that as the preparation was reduces to lyophilized form which increased the stability as compared to conventional liquid formulations. The fluorescence images show the uptake of microparticles by various organs and the ELISA results shows comparable IgG responses along with IgA.

The Chitosan microparticles have higher positive values of zeta potential due to the presence of hydroxyl group of chitosan and shows positive surface charge. In case of charged particles, as the zeta potential increases, the repulsion interaction will be larger leading to the formation of more stable particles with more uniform size distribution. Tap Density is most necessary parameter for aerodynamic microparticles with low density were expected to avoid macrophage uptake and accumulate in deep lung epithellum, one can generate large particles of low density to both optimize the aerodynamic diameter and prevent phagocytosis. Thus, Antibody production was found to be more in pulmonary as compared to other routes. Also it produces systemic humoral responses, but also cell-mediated and mucosal immune responses.
Development and Characterization of Oral Combination vaccine against Hepatitis B & Influenza

Topic: Antiviral and immune therapy for influenza, RSV disease and COVID-19

Nikhil Kapoor (1) S Bhargava (2)

1: GTB Hospital; 2: United Institute of Pharmacy

Vaccination has not only become vital but a lot of revolutionary changes are being observable in the field of vaccine delivery. Vaccine antigens administered by the oral route are often degraded during gastrointestinal transit. Bile salt stabilized vesicles i.e. bilosomes are found to be effective in preventing antigen degradation and enhance mucosal penetration. The aim of the present work was to prepare a combination vaccine system against hepatitis-B (HBsAg) and influenza (r-H1N1Ags). Oral immunization induces both mucosal and systemic immune responses, whereas mucosal responses are not generally observed following systemic immunization. Bilosomes provide needle free, painless approach for immunization, thereby increasing patient compliance and consequently increasing vaccination coverage.

Bilosomes containing HBsAg and r-H1N1Ags were prepared by a lipid cast film method. Antigen loaded bilosomes were characterized in vitro for their shape, size, percent antigen entrapment and stability. Fluorescence microscopy was carried out to confirm the uptake of bilosomes. The in vivo study comprised of estimation of IgG response in serum and sIgA in various body secretions using specific ELISA.

Bilosomes formed were multilamellar and were stable in gastric and intestinal fluids. Fluorescence microscopy suggested that bilosomes were taken up by the gut associated lymphoid tissues. In vivo data demonstrates that bilosomes produced both systemic as well as mucosal antibody responses upon oral administration at higher dose levels as compared to intramuscular immunization but fail to produce any synergistic effect.

Thus, HBsAg potentiates the production anti-r-H1N1 antibody. Also measurable sIgA in mucosal secretions were observed. Thus, the bilosomes are a promising carrier for oral combination vaccines. This approach could be adapted for human use because the mucosal surfaces are the initial sites of infection and it therefore seems logical to attempt to develop vaccination strategies that evoke appropriate localized responses to counteract the early events of pathogenesis.
Benefits of baloxavir in reducing costs of absenteeism: an epidemiological and health economics analysis in influenza

**Topic:** Antiviral and immune therapy for influenza, RSV disease and COVID-19

Rita Freitas (1), Annabelle Lemenuel-Diot (1), Ezgi Anat (1), Gurleen Singh Jhuti (2)

1: F. Hoffmann-La Roche Ltd, Basel, Switzerland; 2: Genentech, Inc., South San Francisco, USA

**BACKGROUND**

Influenza places a significant burden on economies and healthcare systems, one of the reasons being due to it causing substantial absenteeism. We estimated the benefits baloxavir can have on productivity costs, from a Swiss perspective.

**METHODS**

We used a combination of influenza epidemiological modelling and decision tree analysis to estimate baloxavir’s effect on absenteeism.

The epidemiological model consisted of a susceptible, exposed, infectious, recovered (SEIR) model, to estimate the impact of baloxavir’s reduction of time to cessation of viral shedding on the expected number of infected subjects (i.e. reduction in transmission).

The number of infected subjects (treated and untreated) was then entered into the decision tree analysis, populated with branch probabilities andlost earnings as a proxy for absenteeism. We combined these data with hospital length of stay, convalescence data and premature death to calculate the indirect costs incurred by patients with influenza untreated or treated with baloxavir.

This analysis assumed 1 work day costs 286.-CHF, and a population of 8.5 Million inhabitants (66% working). We focused on an otherwise-healthy population and incorporated findings from the baloxavir CAPSTONE-1 trial (NCT02954354) and the literature to estimate the benefits that reducing symptom duration, complications, and disease transmission can have on absenteeism.

**RESULTS**

Deterministic analysis with our decision model estimated a total of 12bCHF lost earnings due to absenteeism or premature death if influenza patients are left untreated. Using baloxavir, we could achieve over 8bCHF savings: -610mCHF by reducing symptom duration (-1.7 days returning to normal health, from CAPSTONE-1 trial), -4.6bCHF if also reducing complications (5% absolute reduction), or up to -8.4bCHF if accounting for reducing transmission in addition to symptom duration and complications (9% absolute reduction).

**CONCLUSIONS**

The use of baloxavir treatment within 2 days of symptom onset in reducing length of symptoms, complications, and transmission of the disease adds up to a considerable economic benefit across the working population of a country.
Weak neutralizing activity of antibodies binding to a conserved region of the coronavirus fusion peptide

**Topic:** Antiviral and immune therapy for influenza, RSV disease and COVID-19

Nathalie Vanderheiden (1), Anneleen Stevaert (2), Jesielong Xie (1), Xiaolei Ren (1), Cyril Barbezange (3), Sam Noppren (2), Isobelle Desombere (4), Bruno Verhasselt (5), Peter Gedhof (6), Nick Vereecke (1, 8), Veere Stroobants (1), Dayoung Oh (1), Merijn Vanhee (8), Lieve Naesens (2), Hans Nauwynck (1)

1: Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium;
2: Rega Institute, KU Leuven, Leuven, Belgium;
3: National Influenza Centre and Epidemiology of Infectious Diseases, Sciensano, Brussels, Belgium;
4: Immune response, Sciensano, Brussels, Belgium;
5: Laboratory for Medical Microbiology, Ghent University Hospital, Ghent, Belgium;
6: Laboratory of Parasitology, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium;
7: PathoSense BV, Lier, Belgium;
8: AZ Delta, Roeselare, Belgium

**BACKGROUND**

Coronaviruses elicit high titers of virus- or variant-specific anti-spike (S) antibodies, many of which bind to the receptor-binding S1 subunit. Identification of conserved epitopes, particularly in the S2 subunit, will help to develop therapeutic antibodies that cover several coronaviruses and variants. We here focused on the fusion peptide (FP) region since it is highly conserved among alpha- and betacoronaviruses.

**METHODS**

We analyzed three sample types: pre-pandemic plasma samples (n=496) from blood donors, sera from hospitalized COVID-19 patients (n=20), taken at different time points post-symptom onset; and a few samples from feline enteric coronavirus (FeCV)-infected cats. Anti-coronavirus antibody titers were determined by (i) immunoperoxidase monolayer assays (IPMA) for SARS-CoV-2 S or N protein, HCoV-OC43, HCoV-229E and FeCV; and (ii) SARS-CoV-2 virus neutralization assays. Serum reactivity towards a part of S2 (e.g. S2Δ cleavage site) FP and heptad repeat 1; SARS-CoV-2 S residues 806-1050) was profiled by pepscan analysis. Peptide-purified antibodies targeting a defined sequence of the FP were isolated from a COVID-19 convalescent serum, using peptide affinity chromatography. These antibodies were analyzed for spike-binding properties using IPMA and surface plasmon resonance (SPR), and for virus-neutralizing activity in SARS-CoV-2-, HCoV-OC43-, or FeCV-infected cells.

**RESULTS**

Antibodies binding to the endemic viruses HCoV-OC43 and HCoV-229E were present in >98% of our pre-pandemic samples and in all our COVID-19 patients at symptom onset. About 50% of the pre-pandemic samples cross-reacted with SARS-CoV-2 S in IPMA assays, however this anti-S response afforded no inhibition in SARS-CoV-2 virus neutralization assays.

Pepscan analyses on the pre-pandemic, COVID-19 and cat sera revealed that a confined region of S2, located at the S2Δ cleavage site and N-terminal helix of the FP, is strongly immunogenic across diverse alpha- and betacoronaviruses. All COVID-19 patients showed a clear increase in serum reactivity towards this N-terminal FP sequence in the 2 to 3 weeks post-symptom onset.

Four peptide-purified human antibodies (pAbs) targeting this short FP region were successfully isolated and confirmed, by SPR, to bind to their cognate peptide as well as to the S2 protein of SARS-CoV-2. The four pAbs efficiently bound to the four tested coronaviruses (i.e. SARS-CoV-2, HCoV-OC43, HCoV-229E and FeCV). This spike-binding capacity was paralleled by a detectable yet transient neutralizing activity in SARS-CoV-2-infected Calu-3 cell cultures.

**CONCLUSION**

Our analysis on sera from pre-pandemic donors, COVID-19 patients and FeCV-infected cats shows that coronavirus cross-reactive antibodies are commonly formed during infection. All three sample types contained antibodies against a defined N-terminal sequence of the spike FP region. These antibodies show broad binding capacity covering alpha- and betacoronaviruses, consistent with the highly conserved nature of their FP epitope. Though exhibiting relatively weak virus-neutralizing activity, the anti-FP pAbs are a relevant starting point to develop anti-coronavirus antibodies offering uniquely broad protection.
Convenient reporter assay to monitor influenza virus HA-mediated fusion, develop fusion inhibitors and determine the HA fusion pH

**Topic:** Antiviral and immune therapy for influenza, RSV disease and COVID-19

Silke Rimaux (1), Annelies Stevaert (1), Ria Van Berwaer (1), Manon Laporte (2), Lieve Naessens (1)

1: Rega Institute, KU Leuven, Leuven, Belgium; 2: Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

**BACKGROUND**

During influenza A virus (IAV) entry, the hemagglutinin undergoes low pH-triggered refolding to induce membrane fusion. Efforts are ongoing to develop small molecule antivirals and anti-HA antibodies that prevent this fusion process. Besides, the pH at which HA is triggered is linked to IAV species adaptation, with human IAVs having a lower fusion pH than avian IAVs. Here we report a robust luciferase reporter-based method to evaluate HA fusion inhibitors and determine the HA fusion pH.

**METHODS**

On day -2, HeLa target cells are transfected in a 96-well plate with two plasmids encoding (i) HA and (ii) pGal4-VP16 (i.e. HSV transactivator VP16 fused to GAL4). In parallel, HeLa overlay cells in a 6-well plate are transfected with pGal5-luciferase reporter plasmid [Pöhlmann, J. Virol., 2011]. One day later, the overlay cells are detached and added to the target cells. On day 0, the cell surface-exposed HA is first activated with trypsin, then briefly exposed to acidic buffer to provoke cell-cell fusion, which results in expression of luciferase that is quantifiable in a luminescence reader. To test potential fusion inhibitors, compound dilutions are added during the acidic stage and to determine the HA fusion pH, the pH of the buffer is varied (range 4.5-5.0).

**RESULTS**

We first optimized all assay variables, i.e. cell density, amount of DNA used for transfection, type of cell detaching reagent and incubation times. Amount of pGal5-luciferase reporter plasmid and its transfection in target cells instead of overlay cells proved to be the most critical factors to achieve reproducible luciferase signals that correlated to the extent of polykaryon formation. When applied to two known inhibitors of H1 HA- or H3 HA-mediated fusion, the reporter method yielded similar EC50 values as obtained earlier by microscopic counting of polykaryons [Vanderlinden, J. Virol. 2010; Leiva, J. Med. Chem. 2018]. In addition, the fusion pH values of several HAs were very similar whether determined by this luciferase reporter method (see Figure) or by microscopy [Laporte, J. Virol. 2019].

![Graph showing pH vs. Fold Increase in Luciferase](image)

**CONCLUSION**

After some optimization, the luciferase reporter-based cell-cell fusion method proved very convenient to quantify HA-mediated fusion and to replace tedious microscopic counting of the polykaryons. The 96-well format enables broad evaluation of influenza virus fusion inhibitors or comparative analysis of different IAV HAs (e.g. from different species) for fusogenic properties.
Measurement of Symptoms in Respiratory Syncytial Virus–Infected Adults: Meaningful Within-Patient Change Thresholds for the Respiratory Infection Intensity and Impact Questionnaire (RIiQ)

**Topic:** Antiviral and immune therapy for influenza, RSV disease and COVID-19

Richard Osborne (1,2), Lauren Nelson (3), Sheri Fehnel (3), Nicole Williams (3), Randall Bender (3), Ryan Ziemiecki (3), Efi Gymnopoulou (4), Els De Paeppe (4), Lindsay Norcross (5), Esther Heijnen (4), Gabriela Ispas (4), Arnaud Cheret (5), Christy Comeaux (6), Benoit Callendret (6), Yannick Vandendijck (4), Eric Chan (7), Jane Scott (8)

1: Measured Solutions for Health, Australia; 2: Centre of Global Health and Equity, Swinburne University of Technology, Australia; 3: RTI Health Solutions, US; 4: Janssen Infectious Diseases, Beerse, Belgium; 5: Janssen, France; 6: Janssen Vaccines & Prevention BV, Leiden, The Netherlands; 7: Janssen Global Services, US; 8: Janssen Global Services, UK

**BACKGROUND**

The Respiratory Infection Intensity and Impact Questionnaire Version 2 (RIiQ™v2) is a patient-reported outcome measure based on the widely used Influenza Intensity and Impact Questionnaire (FluiiQ™) and is designed to assess symptoms and impacts of respiratory syncytial virus (RSV) infection. To facilitate the interpretation of change scores, data from a vaccine trial were used to estimate a range of potential symptom thresholds for the RIiQ™v2 lower respiratory tract (LRT: cough, wheezing, expectoration, shortness of breath) and upper respiratory tract (URT: sore throat, nasal congestion) scores indicating meaningful treatment benefit from the patient perspective.

**METHODS**

At the onset of an acute respiratory infection (ARI Day 1) to ARI Day 29, RIiQ™v2 data were collected electronically daily from 60 confirmed RSV-positive and 1,615 RSV-negative individuals with no coinfections during a phase 2b RSV vaccine trial (NCT03982199). Analyses were conducted using the entire sample pooled across treatment arms. An anchor-based method was applied that used two global measures, patient-reported global impressions of respiratory symptom severity (PGI-S) and health status (PGI-H). Estimates were computed as the mean change in RIiQ™v2 scores among patients improving by one category on each anchor. Two distribution-based methods, the half-standard deviation (half-SD) and standard error of measurement (SEM), were also used to provide a range of threshold estimates.

**RESULTS**

Patterns of mean change in RIiQ™v2 respiratory symptom scores from ARI Day 1 to Day 29 followed the anticipated direction across change in PGI-S and PGI-H groups. Correlations of change between the RIiQ™v2 and global measures were nearly moderate to strong (r ≥ 0.27), supporting the marginal appropriateness of the anchors. Based on the mean change for 1-point improvement PGI-S and PGI-H subgroups, half-SD, and SEM, meaningful within-patient improvement estimates ranged from 0.2 to 0.7 for respiratory symptoms, 0.2 to 0.6 for LRT, and 0.3 to 1.1 for URT (Table 1).

**CONCLUSIONS**

Results present initial estimates for establishing meaningful within-patient change of RIiQ™v2 respiratory symptom scores for use in clinical studies.

<table>
<thead>
<tr>
<th>Method</th>
<th>Meaningful Within-Patient Improvement Thresholds</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>RIiQ™v2 Respiratory Symptom Total</td>
</tr>
<tr>
<td>Anchor-Based (1-point improvement)</td>
<td></td>
</tr>
<tr>
<td>Mean change in PGI-S (ARI Day 1 to 29) n = 157</td>
<td>0.7</td>
</tr>
<tr>
<td>Mean change in PGI-H (ARI Day 1 to 29) n = 164</td>
<td>0.7</td>
</tr>
<tr>
<td>Distribution-based</td>
<td>standard deviation (half-SD)</td>
</tr>
<tr>
<td>Standard error of measurement (SEM)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Table 1. RIiQ™v2 Meaningful Within-Patient Change Improvement Thresholds
Could remdesivir be used in the presence of hepatitis secondary to COVID-19 infection in children? A case report from Iran during the fifth peak

Topic: Antiviral and immune therapy for influenza, RSV disease and COVID-19

Shirin Sayyahfar
Iran university of medical sciences, Iran, Islamic Republic of

BACKGROUND
The pandemic of coronavirus disease 2019 (COVID-19) declared by the World Health Organization (WHO) in March 2020, is still ongoing. At the moment, Iran is experiencing the fifth peak mainly with delta variant. Many children have been involved and despite the previous peaks, the prominent presentation is pneumonia in this population and in comparison to previous peaks of pandemic.

Abnormal liver biochemistry values are common in patients with COVID-19. While more severe cases are more probably associated with hepatitis, a safe, effective and approved drug in this situation is not yet available in pediatric field.

Remdesivir is one of the drugs that has received emergency FDA approval for treatment of covid-19 infection with different results about its efficacy. While there is no approval in pediatric population, we are using it in Iran similar to many countries during this pandemic secondary to lack of any other effective and licensed drug in pediatric field.

One of the side effects of this drug is hepatotoxicity. Therefore, according to the local guideline of Iran ministry of health, before, during and at the end of the treatment, liver function tests (LFTs) should be monitored and when they are five times more than the upper limit of normal values, remdesivir should be withheld.

METHODS
Herein, we report a 6-year-old female with severe acute respiratory syndrome (SARS) coronavirus (CoV)-12B(SARS-CoV-2) pneumonia and hepatitis who was treated with remdesivir despite the first LFTs more than five times the upper limit of normal.

RESULTS
Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) decreased and improved during the course of therapy with remdesivir (table1). This trend continued through the time of patient's hospitalization and almost reached to normal values at the time of discharge (table1).

She was discharged after 14 days of hospitalization and two weeks follow up revealed no complication.

<table>
<thead>
<tr>
<th></th>
<th>First day</th>
<th>Third day</th>
<th>Fifth day</th>
<th>Seventh day</th>
<th>Discharge day</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/L)</td>
<td>186</td>
<td>202</td>
<td>27</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>217</td>
<td>307</td>
<td>16</td>
<td>50</td>
<td>48</td>
</tr>
</tbody>
</table>

Table1 Laboratory characteristics of the patient

CONCLUSIONS
Remdesivir may be used in the presence of hepatitis secondary to COVID-19 infection in children. However, the risk and benefit of remdesivir and possibility of its use when LFTs are elevated at baseline remain to be defined.
**Interferon beta has potent antiviral activity against SARS-CoV-2 including variants of concern**

**Topic:** Antiviral and immune therapy for influenza, RSV disease and COVID-19

Sarah Dudley (1), Aloys Tijsma (2), Victoria Tear (1), Phillip David Monk (1), Stephen Holgate (3), Tom Wilkinson (4,5), Donna Davies (6)

1: Synairgen Research, Southampton General Hospital, Southampton, United Kingdom;
2: Viroclinics Biosciences, Rotterdam, the Netherlands;
3: Synairgen Research, Southampton General Hospital, Southampton, UK; NHRI Southampton Biomedical Research Centre, University Hospital Southampton, Clinical and Experimental Sciences, Faculty of Medicine, University Hospital Southampton, Sir Henry Wellcome Laboratories, Southampton, UK;
4: Clinical & Experimental Sciences, University of Southampton Faculty of Medicine, Sir Henry Wellcome Laboratories, Southampton General Hospital, Tremona Road, Southampton, SO16 6YD, UK;
5: Southampton NHRI Respiratory Biomedical Research Unit, Southampton General Hospital, Southampton, UK;
6: NHRI Southampton Biomedical Research Centre, University Hospital Southampton, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Sir Henry Wellcome Laboratories, Southampton, UK

**BACKGROUND**

Interferon beta (IFN-β) is a naturally occurring protein that is an essential driver of host antiviral responses. The SARS-CoV-2 virus suppresses IFN-β production by cells to evade/compromise the host’s immune responses. SNG001, a formulation of recombinant IFN-β (IFN-β-1a) for inhalation, has been developed as a lung-targeted, host-directed, broad-spectrum, antiviral drug. The aim of treatment is to achieve a high local concentration of IFN-β at the site of infection. Administration of SNG001 directly to the lungs benefited patients hospitalized with COVID-19 in a phase 2 trial. A phase 3 study is ongoing (SPRINTER. NCT04732949). SARS-CoV-2 variants of concern (VOC) are spreading globally; these variants increase transmissibility or enable the virus to evade further the immune system and may have the potential to reduce efficacy of current vaccines and COVID-19 therapeutics. Due to its ability to boost host antiviral responses and its efficacy against SARS-CoV-2, SNG001 was tested for efficacy against SARS-CoV-2 VOC.

**METHODS**

*In vitro* experiments were conducted at Viroclinics-DDL (Rotterdam, Netherlands) to test whether SNG001 shows antiviral activity against the Wuhan-like Germany/BavPat1/2020 strain, and Alpha (B.1.1.7, UK) and Beta (B.1.351), South Africa) VOC. Vero E6 cells were treated with a dilution series of SNG001 16 hours prior to, and retreated after, infection with SARS-CoV-2. Twenty hours after infection, the presence of SARS-CoV-2 viral protein positive cells was determined using immunostaining.

**RESULTS**

SNG001 potently reduced virus to undetectable levels in cells infected with the Germany/BavPat1/2020 strain, and Alpha and Beta SARS-CoV-2 variants. Concentrations, readily achievable following inhaled delivery of interferon beta, that gave 99% inhibition (IC₉₉) were 9.5, 24.7 and 14.8 IU/mL respectively. Further research is being performed on the Gamma (Japan/Brazil) and Delta (India) VOC.

**CONCLUSIONS**

As VOC continue to challenge our ability to control the spread of SARS-CoV-2, lung delivered IFN-β, in the form of SNG001, may become an important SARS-CoV-2 variant-agnostic therapy for hospitalized COVID-19 patients.

Reference

Short-time effect of the 2020/21 season influenza vaccine on RT-PCR-confirmed SARS-CoV-2 infection in a cohort of Italian healthcare workers

Topic: Benefits of vaccinating healthcare workers and other risk groups

Alexander Domnich (1), Andrea Orsi (1,2), Daniele F. Panatto (2), Matilde Oliastro (2), Allegra Ferrari (2), Bianca Bruzzone (1), Laura Sticchi (1,2), Giancarlo Icardi (1,2)

1: Hygiene Unit, San Martino Polyclinico Hospital – IRCCS for Oncology and Neurosciences, Genoa, Italy;
2: Department of Health Sciences (DISAL), University of Genoa, Genoa, Italy

INTRODUCTION

Seasonal influenza vaccination (SIV) is the key public health measure able to reduce the burden of disease and several priority groups (including healthcare workers – HCWs) for annual immunization are well-recognized. The last 2020/21 season northern hemisphere influenza vaccination campaign was carried out during an unprecedented period characterized by a circulation of SARS-CoV-2. In the fear of a possible co-circulation of both influenza viruses and SARS-CoV-2 and the associated increased pressure on healthcare systems, some important policy changes (e.g., mandatory SIV to some groups, including HCWs) took place in some Italian regions. The objective of this study was to investigate the association between 2020/21 SIV and SARS-CoV-2 positivity rate in a cohort of Italian HCWs. Based on some previous reports, we hypothesized some protective non-specific effect (also known as “trained immunity’) of SIV on the RT-PCR-confirmed SARS-CoV-2 infection.

METHODS

In this observational study, the total cohort of HCWs (ca 5000) employed by the San Martino Polyclinico Hospital (Genoa, Italy) was followed retrospectively. The intervention and independent variable of interest was 2020/21 SIV that started on 12th October 2020 and last till mid-January 2021, although most (>50%) doses were administered in October and November 2020. The study outcome was the incidence of SARS-CoV-2 new infections, as determined by RT-PCR. Among potential confounders, the risk of exposure to SARS-CoV-2 was provided as frequency of the SARS-CoV-2 RT-PCR testing. The multivariable Cox regression modelling was applied in order to discern the association of interest.

RESULTS

The final cohort included 2,561 unique HCWs who performed at least one RT-PCR test during the study period; these contributed to a total of 94,438 person-day observations. SIV was administered to 35.6% (95% CI: 33.7–37.5%) of HCWs. Most (62.3% (95% CI: 59.1–65.5%)) HCWs received the quadrivalent cell culture-based vaccine (QIVc; Flucelvax, Seqirus). During the study period a total of 290 new SARS-CoV-2 infections occurred. Overall, the incidence of new SARS-CoV-2 infections was 1.62 (95% CI: 1.27–2.10) and 3.91 (95% CI: 3.43–4.45) per 1,000 person-days in vaccinated and non-vaccinated HCWs, respectively, with a hazard ratio of 0.41 (95% CI: 0.30–0.55). After adjusting, SIV was still a significant predictor (Table 1). Notably, each one more SIV dose was associated with a 15% increase in being tested positive for SARS-CoV-2.

CONCLUSIONS

SIV may exert non-specific protective effects against other infections, likely by reprogramming of innate immune cells and the following immune cascade (i.e., the “trained immunity” hypothesis). However, further observational studies should correct for the risk exposure bias since HCWs more exposed to both viruses may be tested more frequently. In any case, SIV should be actively promoted among all HCWs.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Level</th>
<th>Model 1</th>
<th>P</th>
<th>Model 2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza vaccine</td>
<td>Yes</td>
<td>0.62 (0.54–0.70)</td>
<td>&lt;0.001</td>
<td>0.61 (0.54–0.70)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>1.00 (1.00–1.00)</td>
<td>0.99</td>
<td>1.00 (1.00–1.00)</td>
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<tr>
<td>Age</td>
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<td>1.00 (1.00–1.00)</td>
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<td>1.00 (1.00–1.00)</td>
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<td>Italian</td>
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<td>0.99</td>
<td>1.00 (1.00–1.00)</td>
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<tr>
<td>SARS-CoV2 testing</td>
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<td>0.99</td>
<td>1.00 (1.00–1.00)</td>
<td>0.99</td>
</tr>
<tr>
<td>Consequence standard error</td>
<td>0.62 (0.54)</td>
<td>0.02 (0.01)</td>
<td>0.02 (0.01)</td>
<td>0.02 (0.01)</td>
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</tr>
</tbody>
</table>

Table 1: Multivariable Cox proportional hazard models on the association between 2020/21 influenza vaccination and SARS-CoV-2 first positive test.
Flu vaccination coverage in children with risky pathologies during the 2020-2021 season at eleven health centers

**Topic:** Diagnostic and Intervention strategies for the management of acute respiratory infections

Cristina Giménez Lozano (1), Miriam Escrig Collado (2), Eliseo Pastor Villalba (2)


**BACKGROUND**

Children who have chronic pathologies are a risk group to whom flu vaccination is recommended.

The aim of the study is to evaluate flu vaccination coverage in children aged 0 to 15 years with risky pathologies during the 2020-2021 season at eleven health centers of department Valencia-Peset (with an assigned population of 35376 children up to 15 years) and to assess the differences found based on age group, sex, health center and pediatrician.

**METHODS**

Observational, retrospective and descriptive study with data on childhood flu vaccination coverage (children up to 15 years) obtained from the Vaccine Information System (SIV) of the Valencian Community. Coverage was analyzed by age groups, sex, health center and pediatrician.

**RESULTS**

During 2020-2021 influenza vaccination season, 1173 out of 3335 children with risk conditions were vaccinated, and global coverage was 35.17% [IC 95%: 33.55-36.82%].

Statistically significant differences in vaccination coverages were observed when divided by age groups: children aged 5-9 years showed the highest coverage (38.46%), followed by children aged 10-14 years (36.75%) and those aged 0-4 years (29.81%) (Graph 1).

No statistically significant differences were found in vaccination coverages between boys (35.01%) and girls (35.38%).

By health center the vaccination coverage range was 16.08-60.14% (Graph 2). Important differences in coverage were also observed depending on the pediatrician in each health center (coverage range by pediatrician and health center is showed in Table 1).

**CONCLUSIONS**

Influenza vaccination coverage in children with underlying diseases is low, being slightly higher in the 5-9 years group. There is variability not only between health centers but also between pediatricians from the same health center. The implementation in primary care of influenza vaccination in pediatric children with risk pathologies must be improved.

**Keywords** Vaccination; Coverage; flu; pediatricians.

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<table>
<thead>
<tr>
<th>Health center</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altafaro</td>
<td>13.46%</td>
<td>18.18%</td>
</tr>
<tr>
<td>Sadavi</td>
<td>15.25%</td>
<td>41.00%</td>
</tr>
<tr>
<td>Ruzafa</td>
<td>19.77%</td>
<td>53.19%</td>
</tr>
<tr>
<td>Castellar</td>
<td>20.00%</td>
<td>23.53%</td>
</tr>
<tr>
<td>Ing. Joaquin Benlloch</td>
<td>21.10%</td>
<td>33.73%</td>
</tr>
<tr>
<td>Fuente San Luis</td>
<td>23.44%</td>
<td>67.47%</td>
</tr>
<tr>
<td>San Marcelino</td>
<td>35.21%</td>
<td>37.28%</td>
</tr>
<tr>
<td>Plaza Segovia</td>
<td>36.21%</td>
<td>40.91%</td>
</tr>
<tr>
<td>Parera Jofré</td>
<td>41.35%</td>
<td>61.02%</td>
</tr>
<tr>
<td>Beneüasser</td>
<td>50.00%</td>
<td>66.16%</td>
</tr>
</tbody>
</table>

Table 1. Flu vaccination coverage range by pediatricians in each health center. Only those pediatricians with at least 10 assigned patients have been considered.
Graph 1. Flu vaccination coverage by age group and sex
(children up to 15 years)

Graph 2. Global coverage by health center
Flu vaccination during six seasons (2015-16 to 2020-21) in the Valencia-Clínico department of the Valencian Community

**Topic:** Diagnostic and Intervention strategies for the management of acute respiratory infections

Cristina Giménez Lozano (1), Miriam Escrib Collado (2), Carolina Blanco Calvo (1), Eliseo Pastor Villalba (2)

1: Servicio de Medicina Preventiva, Hospital Doctor Peset, Valencia, Spain;  
2: Servicio de Promoción de la Salud y Prevención en las Etapas de la Vida, Dirección General de Salud Pública y Adicciones, Valencia, Spain

**BACKGROUND**

The COVID-19 pandemic may have caused an increase in adherence to influenza vaccination in risk groups. The main objective is to assess the number of immunizations against influenza in the Valencia-Clínico department (with an assigned population of 339,896 inhabitants) of the Valencian Community (VC) during six seasons (from 2015-16 to 2020-21) and to analyze the differences based on age, season and risk group.

**METHODS**

A descriptive, observational and retrospective study of influenza immunizations carried out in the Valencia-Clínico department of the VC during six seasons (2015-16 to 2020-21) was performed. The data was extracted from the Vaccine Information System-Nominal Vaccine Registry (SIV-RVN) and examined by season, age group, sex and risk group.

**RESULTS**

In the Valencia-Clínico department, along the six seasons analyzed, 319,350 immunizations against influenza have been carried out, of which 59.81% were in the ≥64 years group, 34.95% in the 15-64 years group and 5.53% in those aged <15 years. 56.09% of immunizations were carried out in women. By risk groups: the majority were patients with chronic cardiovascular and respiratory diseases with 48.16% of immunization throughout the period.

The total number of immunizations has been increasing since the 2015-16 season. During the seasons prior to the start of the SARS-CoV-2 pandemic, the greatest increase occurred in 2019-2020, when it increased 5.9% compared to the previous campaign. However, the greatest increase has been experienced in the last season 2020-21, already concurring with the pandemic, with an increase of 59.9% in the number of people immunized compared to the previous season.

By age groups, during the last season 2020-21 the greatest increase occurred in the ≤15 years group with a percentage increase of 111.3% (Graph 1).

By risk group, during the last season 2020-21, the highest increase was observed in people aged over 64 without any other risk factor and in healthcare workers and special public services (93.9% and 90.8% respectively, as shown in Graph 2).

**CONCLUSIONS**

Despite the fact that influenza immunization has followed an upward trend since 2015, it was during the last 2020-21 season that it experienced the greatest increase. The COVID-19 pandemic has influenced this increase, with a greater impact on people aged under 15 years with risk factors, health workers and caregivers, groups that traditionally had low vaccination coverage.

**Keywords:** Flu vaccination, COVID-19, coverage
Graph 1. Evolution of the number of hospitalizations during the 2015-2021 seasons by risk group
Flu vaccination coverage in adults during 2020-2021 season at 11 health centers

**Background**

Flu vaccination is recommended for people with underlying diseases and for those aged over 65 years. The aim of the study was to assess the flu vaccination coverage in adults over age 14 to whom flu vaccination is recommended at 11 health centers of Department Valencia-Peset (with an assigned population of 237,810 adults in December 2020) during 2020-2021 season, and to analyze risk group, sex and health center differences.

**Methods**

An Observational, retrospective, and descriptive study with data obtained from the Vaccine Information System (SIV) of the Valencian Community was performed. Flu vaccination coverage in adults over age 14 from 11 health centers of Department Valencia-Peset was obtained and analyzed by risk groups, sex, age and underlying pathologies.

**Results**

During 2020-2021 season, 38,441 adults were vaccinated out of 86,164 who had risk conditions or underlying pathologies, and global coverage was 44.61% [IC 95% 44.28-44.95].

By risk group, 64.52% of adults over 65 with underlying pathologies, 44.36% of adults over 65 without underlying pathologies and 26% of adults under age 65 were vaccinated. These differences were statistically significant.

By sex, women presented greater coverage (45.84%) than men (43.03%). The only subgroup in which there was greater coverage in men was in adults over 65 with underlying pathologies (Graph 1). These differences were statistically significant.

By health center, the coverage range was 40.18-52.55% (Table 1).

**Conclusions**

Flu vaccination coverage continues to be low, even among adults aged over 65, in which it has not been possible to achieve the 75% coverage defined as a target during the 2020-2021 season in the Valencian Community. Coverage in adults over age 65 with underlying pathologies is higher than in those without pathologies.

**Keywords** Flu vaccination; Coverage; Adults

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**Graph 1. Flu vaccination coverage by risk group and sex (adults over 14 years)**

**Table 1. Flu vaccination coverage by health center and risk group (adults over 14 years)**
SARS-COV-2 TEST TURNAROUND TIMES IN A PUBLIC DIAGNOSTIC SERVICE LABORATORY, GAUTENG SOUTH AFRICA, 2020

Topic: Diagnostic testing in the management of acute respiratory infections in primary and secondary care

Beauty Kalenga (1), Avani Bharuthram (2), Ashlyn Davis (1), Andiswa Simane (1), Bulelani Manene (1), Kathleen Subramoney (2), Bonolo Mashishi (2), Nonhlanhla Mbenenge (2), Florette Treurnicht (2)

1: Department of Virology, National Health Laboratory Service, Parktown, South Africa; 2: Department of Virology, National Health Laboratory Service and University of the Witwatersrand, South Africa

BACKGROUND

Severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) test results are perceived to have been delayed to such an extent during the 2020 pandemic that clinical management and public health interventions were compromised. Here, SARS-CoV-2 test turnaround times (TATs) were analysed in the Virology laboratory, Charlotte Maxeke Johannesburg Academic Hospital, Gauteng, South Africa in 2020.

METHODS

From 16 March to 18 September 2020 respiratory samples received as dry swabs were placed in phosphate buffered saline. Total nucleic acids or crude lysates were prepared. SARS-CoV-2 infection was identified through real-time reverse transcription polymerase chain reaction assays. The TATs is the time between the sample collection and test result dates.

RESULTS

A total of 326,377 respiratory samples were tested. 67.8% (221,487/326,377) were from community screening. The “clinical” samples defined mainly as inpatients represented 32.1% (104,890/326,377). Fifty-four percent of clinical samples (56,851/104,890) were resulted within 3 days (average 1.7 days), 72.2% (75,704/104,890) within 7 days (average 2.6 days) and 83.8% (87,859/104,890) within 14 days (average 3.7 days). In comparison, 65.2% (146,614/221,487) of community samples were resulted within 14 days (average 6 days), 46.0% (101,990/221,487) within 7 days (average 4.0 days) and 19.8% (43,955/221,487) within 3 days (average 2.2 days).

Overall SARS-CoV-2 positivity was 11.6% (37,771/326,377). Clinical and community samples had similar positivity rates for TAT <14 days; 14.8% and 14.6% respectively. Tests resulted ≥14 days had higher positivity rates among community compared to clinical samples.

CONCLUSION

Inpatients’ samples were prioritised for TAT of 24 hours with an average of 2-3 days whereas longer TATs were seen among community samples with 45% resulted within 4 days. We conclude that in general TATs for SARS-CoV-2 complied with requirements to facilitate clinical management and public health actions.
MOLECULAR GENETIC ANALYSIS OF SARS-COV-2 LINEAGES IN ARMENIA

Topic: Diagnostic testing in the management of acute respiratory infections in primary and secondary care

Gayane Melik-Andreadyan (1), Shushan Sargsyan (1), Armine Ghazazyan (1), Naira Akselyan (1), Diana Avetyan (2,3), Siras Hakobyan (2), Arsen Arakelyan (2,3), Lilit Avetisyan (1), Artsavaz Vanyan (1)

1: National Center of Disease Control and Prevention Ministry of Health (NCDCP), Armenia;
2: Institute of Molecular Biology National Academy of Sciences RA;
3: Russian Armenian University, Yerevan, Armenia

BACKGROUND
Sequencing of SARS-CoV-2 provides essential information into viral evolution, transmission, and epidemiology. Here we performed whole-genome nanopore sequencing and molecular-genetic characterization of SARS-CoV-2 from clinical specimens, conducted lineage and phylogenetic analysis aimed at identification of the most prevalent lineages at different time points and understanding the transmission routes of the virus to Armenia.

METHODS
Thirty-six RNA samples were selected from batches of COVID-19 positive samples tested at National Center for Disease Control and Prevention MH RA on 22nd and 29th January, and 18 March 2021 (twelve samples per day). Nanopore sequencing was performed according to “nCoV-2019 sequencing protocol v3 (LoCost) V3” with ARTIC V3 primer scheme. Base calling and demultiplexing were performed using Guppy. Downstream analyses were performed using the minimap/nanopolish workflow implemented in the ARTIC pipeline. The hCoV-19/Wuhan/WIV04 was used as a reference. Variant analysis and functional annotation were performed using the CovSurver, CoV-GLUE, and CorGAT tools. Multiple alignment was performed using MAFFT (v7) tool. A phylogenetic tree was built using “dist.alignment” and “phylogram” R packages. Clade and lineage analysis was performed using the PANGOLIN.

RESULTS
PANGO lineage analysis assigned January samples as follows: twenty-one to B.1.1.163 lineage; 2 samples were assigned B.1.1.208 and 1 sample was assigned to B.1.1 lineage. The B.1.1.163 lineage was the most frequent in Russia, which was the first country Armenia opened borders with. The lineage composition has been changed dramatically in clinical samples collected in March. PANGO analysis assigned all sequences to B.1.1.7 Alpha (UK) lineage with characteristic N301Y, A570D, P681H, and D677G stop mutations found in all samples.

Phylogenetic analysis of B.1.1.163 lineage sequences has placed samples collected in January in the cluster along with sequences from Europe and Russia suggesting the possible single transmission route for this lineage. In contrast, B.1.1.7 Armenian sequences were clustered on different phylogenetic tree branches indicating multiple routes of transmission.

The results of the analysis showed that Charité RdRP primers/probes, NID N gene and US CDC N3 primers contained the highest number of mutations. N3 forward primer mutations located in 5′ regions and did not influence the primer binding since we obtained the N gene PCR signal in all studied samples.

CONCLUSIONS
The results of the study emphasize the need for constant sequencing-based surveillance of SARS-CoV-2 strains for public health decision-making and can play an important role in shaping local, national, and regional COVID-19 response strategies. Nanopore sequencing can serve as an efficient and affordable alternative for epidemiologic surveillance and molecular-genetic analyses of SARS-CoV-2. This is particularly important in countries with underdeveloped NGS infrastructure, such as Armenia.
Impact of integrating routine, syndromic molecular point-of-care testing for SARS-CoV-2 and other respiratory viruses into an acute oncology service

**Topic:** Diagnostic testing in the management of acute respiratory infections in primary and secondary care

**Kate Beard (1,2), Florina Borca (3), Hang T.T. Phan (3), Alex Tanner (1), Nathan Brendish (1,2,4), Tristan Clark (1,2,4)**

1. Department of Infection, University Hospital Southampton NHS Foundation Trust, Southampton, UK;
2. School of Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK;
3. Clinical Informatics Research Unit, University of Southampton, Southampton, UK;
4. NHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust, Southampton, UK

**BACKGROUND**

The COVID-19 pandemic has caused significant challenges for infection prevention measures and patient flow within hospital acute admission pathways. The long turnaround times associated with laboratory PCR testing compounds these challenges. An Acute Oncology Service (AOS) is a key hospital admission pathway recommended in all UK hospitals with emergency departments. We aimed to assess the clinical impact of replacing laboratory PCR with routine molecular point-of-care testing (mPOCT) for SARS-CoV-2 and other respiratory viruses, in an AOS.

**METHODS**

This pre and post-implementation study took place in the AOS of a large teaching hospital, in Southampton, UK. We collected patient data from two periods: November 25th 2019 to November 24th 2020, when respiratory virus testing took place using the on-site laboratory and December 1st 2020 to May 31st 2021 following the introduction of mPOCT using the QIAstat-Dx Respiratory SARS-CoV-2 Panel, located in the AOS unit. The primary outcome was the time to results being available.

**RESULTS**

Between 25th November 2019 and 24th November 2020 2189 patients (Aged ≥18 years) admitted to the AOS unit were tested for SARS-CoV-2 and/or other respiratory viruses via centralised laboratory PCR testing on admission. Between 1st December 2020 and 31st May 2021 1540 patients admitted to the AOS unit received mPOCT. Baseline characteristics were similar between the groups. Median (IQR) time to results was 5.8 h (4.2 to 10.6) in the pre-implementation period and 1.9 h (1.9 to 3.0) in the post-implementation period. (difference -3.9 h [95% CI to -3.8 to -3.5]; p=0.0001). The median (IQR) time spent in assessment areas was 6.0 h (4.1 to 7.5) in the pre-implementation period and 5.5 h (3.8 to 7.4) in the post-implementation period. (difference -0.5h [95% CI=0.5 to -0.2]; p=0.0001). The median (IQR) time from radiology request to results, was 2.5 h (1.1 to 7.4 IQR) in the pre-implementation period and 1.9 h (0.9 to 4.6 IQR) in the post-implementation period (difference -0.6 h [95% CI -0.6 to -0.2]; p=0.0001). 30.0% (656/2188) of patient episodes underwent same-day discharge from the AOS unit in the pre-implementation period, and 35.3% (543/1540) in the post-implementation period (relative risk 0.85 [95% CI 0.77 to 0.93]; p=0.0007). 20/2183 (0.9%) of patients admitted to hospital via AOS had a hospital-acquired respiratory virus infection (HAI) detected on an oncology ward during the pre-implementation period versus 0/1182 (0%) in the post-implementation period (difference of 0.9% [95% CI=0.4 to 1.4 ]; p=0.0002, number needed to test to prevent one HAI=139).

**CONCLUSIONS**

Introducing routine syndromic mPOCT for respiratory viruses including SARS-CoV-2, was associated with a reduced time to results, compared to laboratory PCR testing. mPOCT was also associated with reduced time in assessment areas, reduced time to radiology results and a reduction in the rates of hospital acquired respiratory virus infection.
The Secondary Care Burden in England of Influenza Related Admissions in Patients with Diabetes 2017-2020

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Craig Davidson, Ilana Gibbons, Orsolya Balegh, Babis Valmas
Sanofi Pasteur, United Kingdom

**BACKGROUND**
The Quality and Outcomes Framework data suggests that over 1 million people with diabetes did not receive an influenza vaccination in 2019/20 in England. Influenza vaccination can reduce complications, and the National Flu Immunisation Programme recommends annual vaccination for people with diabetes, yet the vaccination coverage rate in England of 61.2% remains below the WHO recommended rate (75%). Using Hospital Episode Statistics (HES) data we sought to analyse the direct secondary care burden of coded influenza cases in England in people with diabetes in the 2017 to 2020 seasons.

**METHODS**
Using HES data (September-March; 2017-2020 seasons) all non-elective admissions, patient counts, bed days, tariff costs with primary or secondary diagnosis ICD-10 codes related to influenza (J09-J11) and diabetes (E10-E14) were extracted. Admissions and patient numbers were rounded up to the nearest 5 in accordance with NHS Digital guidelines. From these data we calculated national mean length of stay (LOS) and cost per admission.

**RESULTS**
Over the three years, there were 21,280 non-elective spells for influenza admissions in patients with diabetes. This cost £75.566.200 in non-elective inpatient costs. Average length of stay was >10 days in all years, and average non-elective cost of admission ranged between £3,580-£4,061. The population admitted with diabetes and influenza tend to be older with average ages between 68-71 years. 67% of patients admitted were >65 years. J09 (Influenza) was the most common primary ICD-10 code in all years, and WJ2 (Standard Infectious Diseases without Interventions) was the most common HRG tariff.

**CONCLUSION**
Influenza in patients with diabetes can have a substantial, negative impact on the healthcare system even before considering primary care and broader societal burdens. Prioritising prevention of influenza through vaccination, and the use of more effective vaccines, particularly in the over 65-year olds, could result in considerable savings for the healthcare system and alleviate temporal pressures on hospital resources.

This study was funded by Sanofi Pasteur

**References**
https://gpcontract.co.uk/browse/UK/Diabetes%20Mellitus/20 (Accessed 16/06/2021)
COVID-19 infections in a cohort of Canadian healthcare personnel

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

**Background:**
SARS-CoV-2 has caused more than 4 million deaths within the first 18 months of the pandemic. Healthcare personnel (HCP) are at the front lines during pandemics, putting themselves and their families at risk of infection. This cohort study of Canadian HCP describes the impact across several acute care sites and provinces during the first year of the pandemic.

**Methods:**
HCP were recruited to a cohort study starting June 15, 2020. They completed an online baseline survey at enrolment, and reported potential exposures (work and community), and testing for COVID-19 (i.e., respiratory sample tested using polymerase chain reaction (PCR)) throughout the pandemic. They could volunteer to provide blood samples (either serum or dried blood spot) to assess IgG antibody levels (to nucleoprotein, receptor-binding domain, and spike trimer) at various time points. Logistic regression was used to assess risk factors for infection.

**Results:**
Of the 1795 consenting HCP, 1787 (99.6%) completed a baseline questionnaire, 821 reported ≥1 test for COVID-19, and 1004 provided ≥1 blood sample. The median age of participants was 40 years (range 18-74), 84.8% were female, and 34% were nursing staff. Of the 1787 participants, 106 HCP (5.9%) tested positive; 97 by PCR and 9 by antibody. Of these, 99 (5.5%) tested positive prior to vaccination against COVID-19 while 7 had breakthrough infections (0.6% of 1101 HCP with ≥2 doses). The breakthrough infections were diagnosed by PCR and occurred 64 to 146 days after their second dose.

Risk factors for infection prior to being vaccinated included household size (odds ratio (OR) 1.21; 95% confidence interval (CI) 1.04-1.42 per additional person), close contact (within arm's length for ≥2 minutes) with a non-household member (peer, patient, or other) with confirmed COVID-19 (OR 2.62; 95%CI 1.52-4.50), number of close contacts with non-household members (OR 1.04; 95%CI 1.01-1.07 per additional person), and working in a higher risk hospital area (OR 1.55; 95%CI 1.00-2.43) defined as working ≥20 hours/week in an emergency department, adult intensive care unit, adult medical ward, or a COVID-19 assessment centre.

**Conclusion:**
Although the incidence of HCP infection was not higher than that in the general population, working in higher risk hospital areas was associated with increased risk of infection.
High-throughput Mutational Surveillance of the SARS-CoV-2 Spike Gene

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Ulrich Elling
Institute of Molecular Biotechnology (IMBA), Austria

**BACKGROUND**
SARS-CoV-2 has evolved rapidly towards higher infectivity and partial immune escape over the course of the pandemic. This evolution is driven by the enormous virus population, that has infected close to 200 million people by now. Therefore, cost-effective and scalable methods are needed to monitor viral evolution globally.

**METHODS**
Mutation-specific PCR approaches have become inadequate to distinguish the variety of circulating SARS-CoV-2 variants and are unable to detect novel ones. Conversely, whole genome sequencing protocols remain too labor- and cost-intensive to monitor SARS-CoV-2 at the required density. By adapting SARSeq we present a simple, fast, and scalable S-gene tiling pipeline for focused sequencing of the S-gene encoding for the spike protein.

**RESULTS**
This method reports on all sequence positions with known importance for infectivity and immunity, yet scales to >20K samples per run. S-gene tiling is used for nationwide surveillance of SARS-CoV-2 at a density of 10% to 50% of all cases of infection in Austria. We show that SARSeq S-tiling achieves full coverage of the region of interest in samples with Ct values as high as 33-35 and can reliably detect known and identify novel variants present in as little as 1% within one sample. We also benchmarked SARSeq S-tiling against whole genome sequencing (WGS) and further provide a simple bioinformatic pipeline to report sample-based results. SARSeq S-tiling uncovered several infection clusters with variants of concern and successfully informed public health measures in a timely manner, allowing their successful implementation.

**CONCLUSIONS**
S-gene tiling using SARSeq represents a rapid, sensitive, and cost-effective method for surveillance of SARS-CoV-2 variants. It is used for surveillance of SARS-CoV-2 in Austria and uncovered e.g. the biggest cluster of B.1.351/Beta outside Africa, that was situated in Tyrol, as well as the E484K mutation in Alpha, that also occurred in Tyrol. Further, our close monitoring of mutations further highlighted evolutionary constraints and freedom of the spike protein ectodomain and sheds light on foreseeable evolutionary trajectories, that are of high relevance for vaccination strategies.
Keeping Up with The Variants: SARS-CoV-2 Evolution Drives Change in Evidence Generation

Topic: Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Anthony Marchese, Hadi Beyhaghi
Novavax, Inc., United States of America

BACKGROUND

As new SARS-CoV-2 variants emerge, the landscape of variant-specific evidence generation on COVID-19 vaccine effectiveness continues to evolve. We characterized evidence generation efforts to assess the effectiveness of select COVID-19 vaccines against variants of concern (VOC) with respect to speed of reporting, evidence type, study locations, and sponsorship information.

METHODS

We conducted a targeted review of online sources, including press releases, pre-print manuscripts, and peer-reviewed articles, which identified alpha, beta, gamma, and delta variant-specific vaccine effectiveness data. For each variant, we extracted and recorded the earliest date of laboratory evidence based on neutralizing antibody titers from sera of vaccinated individuals, clinical trial evidence, and real-world evidence (RWE) along with study locations, results, and sponsorship information for COVID-19 vaccines from Pfizer, Moderna, Janssen, AstraZeneca, and Novavax.

RESULTS

The earliest delta-specific laboratory data were reported one day prior to the delta variant’s VOC designation, while the corresponding data for alpha, beta, and gamma variants became available 21 days, 38 days, and 37 days after each variant’s VOC designation, respectively. Similarly, delta-specific RWE on vaccine effectiveness became available 13 days post-VOC designation, while alpha- and beta-specific RWE were first reported 83 and 138 days after the VOC designation, respectively. RWE were predominantly government-funded test-negative case-control studies conducted in regions with advanced research infrastructure and high vaccine coverage such as the UK. Clinical trial evidence for vaccine efficacy was first reported 41 days after VOC designation for alpha and beta variants. These trials were being conducted serendipitously in the regions where specific variants first emerged and became the dominant variant at the time of conducting the clinical trials e.g., the UK and South Africa for alpha and beta variants, respectively. The figure below summarizes the first reported vaccine effectiveness evidence for VOCs.

CONCLUSIONS

Compared to other VOCs, the speed with which delta-specific evidence were generated suggests a faster evidence generation response to emerging variants, with laboratory data typically being the first type of evidence to be reported. In the absence of widely accepted correlates of protection using laboratory data and completion of pivotal clinical trials evaluating vaccine efficacy, ongoing real-world studies, particularly test-negative case-control design, can provide an efficient and reliable source of vaccine effectiveness evidence against emerging SARS-CoV-2 variants. Expansion of government- and manufacturer-sponsored real-world studies in different regions can help inform public health policy in response to emerging VOCs.
The epidemiology of COVID-19 in Ontario elementary and secondary school education workers: an interim analysis following the first school year

**Objective**
To estimate the cumulative incidence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in education workers and the factors associated with testing positive to July 2021.

**Methods**
A prospective cohort study of education workers working ≥8 hours per week during the 2020-2021 school year in the province of Ontario, Canada. Participants self-reported results of tests for SARS-CoV-2 and completed online surveys about demographic information, exposures, and vaccinations against SARS-CoV-2. Blood samples to assess anti-SARS-CoV-2 antibodies were self-collected at enrolment and prior to and/or 30 days after vaccination using dried blood spot collection kits. Multivariable regression was used to assess risk factors for infection.

**Results**
Of the 2834 participants, 85% were female, 81% were teaching staff, 86% had received at least one dose of SARS-CoV-2 vaccine, and 70% had ever been tested for SARS-CoV-2 using respiratory specimens. Of those who had been tested via a respiratory specimen, 3.5% reported a positive test. Another 5 participants tested positive according to their blood sample (3.6% overall incidence). In multivariable regression analysis, risk factors for infection included exposure to an adult in the household (adjusted incidence rate ratio (aIRR) 12.4; 95% confidence interval 7.8, 19.8), a child in the household (aIRR 2.6; 1.4, 4.8), or a student at school (aIRR 1.9; 1.1, 3.1), or travel (aIRR 5.6; 1.2, 25.6).

**Conclusion**
Education workers have a similar risk of infection with SARS-CoV-2 as other Ontario residents. Practicing protective measures when exposed to sick people within the household and at all times when exposed to coworkers, students, or anyone else, including while travelling, would help reduce the risk of infection.

Topic: Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Ángel Gil (1), Javier Díez-Domingo (2), Federico Martín-López (3), Esther Redondo (4), Raúl Ortiz de Lejarazu (5), Jaime Correia de Sousa (6), Carlos Rabaçal (7), João Raposo (8), Carlos Robale Cordeiro (8), Mafalda Carmo (9), Hugo Lopes (11), José María Guillén (12), Catarina Gomes (13), Margarida Martins (13), Carlos Guzman (12), Hélène Bricout (14), Filipe Froes (15)

1: Public Health and Medical Specialties Department, Health Sciences Faculty, Juan Carlos University, Madrid, Spain;
2: Vaccine Research Department, University of Valencia, Valencia, Spain;
3: Paediatric Department, Hospital Clínico Universitario de Santiago, Santiago de Compostela, Spain;
4: International Health Center Madrid Health, City Council of Madrid, Madrid, Spain;
5: Valladolid National Influenza Centre, Hospital: Clínico Universitario de Valladolid, Valladolid, Spain;
6: Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga Portugal. ICVS/IBS’s. PT Government Associate Laboratory, Braga/Guimarães, Portugal;
7: Vila Franca de Xira Hospital, Lisbon, Portugal;
8: AMPHP and Nova Medical School, Lisbon, Portugal;
9: Pulmonology Department, Coimbra University Hospital, University of Coimbra, Coimbra, Portugal;
10: IKBF, Barcelona, Spain;
11: IKBF, Lisbon, Portugal;
12: Sanofi Pasteur, Madrid, Spain;
13: Sanofi Pasteur, Lisbon, Portugal;
14: Sanofi Pasteur, Lyon, France;
15: ICU, Thorax Department, Centro Hospitalar Universitário Lisboa Norte, Lisbon, Portugal.

BACKGROUND
Spain and Portugal have seasonal influenza vaccination programmes covering high-risk groups, such as those aged ≥65 years or with specific medical conditions. The goal of this study was to describe the burden of hospitalizations attributable to influenza in each country, over 10 past seasons.

METHODS
Data were extracted from administrative databases from 2008-2018, which contain all National Health Service (NHS) hospitalization records in mainland Portugal and approximately 60% of NHS hospitalization records in Spain, which are then extrapolated to the whole country. Influenza episodes were defined as those coded with ICD-10 J09, J10 or J11 in any primary or secondary diagnosis field. Mean results for the study period exclude the season 2009/10 H1N1 pandemic.

RESULTS
Mean annual number of hospitalizations per season was 13,063 in Spain and 1,207 in Portugal. A total of 121,393 and 13,629 influenza hospitalizations were identified over the 10 seasons in Spain and Portugal, respectively with a mean hospitalization rate per 100,000 inhabitants of 28.1 for Spain and 11.6 for Portugal. The peak of annual hospitalizations was observed in season 2017/18 both in Spain (37,619) and Portugal (3,007). Patients aged ≥65 years contributed to 56.7% of influenza hospitalizations in Spain and 46.2% in Portugal. Patients with comorbidities accounted for 59.0% of hospitalizations in Spain and 65.6% in Portugal. The mean in-hospital mortality rate per season in Spain was 5.2%, increasing to 7.0% in patients aged ≥65 and to 6.3% among patients with comorbidities. In Portugal, those rates were 6.1%, 9.5% and 8.2% respectively. The mean length-of-stay (LOS) in Portugal was 12.0 days, increasing to 12.4 days in patients with comorbidities. The results for Spain showed 9.4, 9.8 and 10.9 days for the respective groups of patients.

CONCLUSIONS
The burden of severe influenza is substantial, especially in high-risk groups. Higher influenza hospitalization rates were observed in Spain, which is consistent with differences in weekly incidence rates of influenza-like-illness estimated by national surveillance systems. Spain presented lower LOS and in-hospital mortality. It is unclear whether these variations reflect true geographic variation or are due to testing, coding, or other clinical practice differences. In both countries, experts perceive an increase in influenza testing at admission, which can contribute to the recent growth of reported cases.

Funding This study was funded by Sanofi Pasteur.
Assessment of seroprevalence to SARS-COV-2 among blood plasma donors during the pre-vaccination stage of the COVID-19 pandemic in Ukraine

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Tetyana Chumachenko (1), Maryna Baseva (2), Lyubov Makhota (3), Galina Ostapenko (1), Dmytro Chumachenko (4)

1: Kharkiv National Medical University, Ukraine;
2: LLC «Kharkiv Regional Center of Plasma’s Provision And Processing «Kharkiv-Plasma», Ukraine;
3: Kharkiv Regional Center for Prevention and Control Diseases of the Ministry of Health of Ukraine, Ukraine;
4: National Aerospace University “Kharkiv Aviation Institute”, Ukraine

**BACKGROUND**

COVID-19 is characterized by a polymorphism of clinical symptoms from an asymptomatic and mild course of the disease to severe and fatal cases. The number of reported COVID-19 cases does not reflect the real epidemic situation. Assessment of seroprevalence to the SARS-CoV-2 helps to inform how the virus spreads among the population and also informs forecasts of the dynamics and patterns of the epidemic process.

This work aimed to assess the seroprevalence to the SARS-CoV-2 virus among blood plasma donors in the Kharkiv oblast (Ukraine).

**METHODS**

A cross-sectional epidemiological study of seroprevalence to the SARS-CoV-2 was carried out among blood plasma donors from May 6, 2020, to March 16, 2021. Blood plasma samples were received from 6399 donors. Among them, there were 4035 (63.1%) men and no vaccinated persons.

The age of the participants ranged from 18 to 72 years, mean was 29.5 years, mode was 19 years. The level of antibodies against the nucleocapsid antigen was tested by the electrochemiluminescence method.

**RESULTS**

In the Kharkiv oblast, the first COVID-19 case was registered in March 2020. The number of cases increased to 248 in April 2020, to 817 in May, in June there was a slight increase in the incidence to 852 cases, after which the incidence began sharply grow, and 19,044 cases were reported in November 2020. Within three months, the number of cases detected per month ranged from 14,036 in December 2020 to 5873 in February 2021. In March 2021, the maximum number of cases was registered (19989 COVID-19 patients).

We found out that the seroprevalence to the SARS-CoV-2 among donors was 2.2% in May and 1.8% in June 2020. In the following months, there was a significant increase in the indicator, and in January 2021 it reached 53.8%. In February 2021 and March 2021, there was a slight decrease in the proportion of donors in whose plasma specific antibodies were determined and was 48.9% and 46.4% in February 2021 and March 2021, respectively.

**CONCLUSIONS**

The growth of the seroprevalence to the SARS-CoV-2 among blood plasma donors is the marker of an increase in the spread of the SARS-CoV-2 among donors, the majority of whom are the young adult population of the Kharkiv oblast, indicating the intensity of the COVID-19 epidemic process. Herd immunity is formed gradually and has not yet reached the level necessary to curb the circulation of the pathogen. Because of our limited understanding on the duration of humoral immunity to COVID-19, and our challenges with monitoring the circulation of the pathogen toward herd immunity, it remains important to study seroprevalence dynamics.
Impact of the COVID-19 pandemic on respiratory infectious diseases in the UK and Ireland

Topic: Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Heena Odedra (1), Lorraine Glynn (2); Craig Davidson (1)

1: Sanofi Pasteur, Reading, United Kingdom; 2: Sanofi Pasteur, Dublin, Ireland

BACKGROUND

Influenza and respiratory syncytial virus (RSV) cause seasonal respiratory tract infections (RTIs) that in temperate climates peak during the winter (typically October to May for influenza and October to March for RSV). (1-7) The emergence of COVID-19 and subsequent non-pharmaceutical mitigation measures have caused widespread social disruptions that have affected transmission and circulation patterns of seasonal respiratory viruses. (1, 8)

OBJECTIVES

To describe the impact of non-pharmaceutical COVID-19 mitigation measures on influenza and RSV activity in the UK and Ireland.

METHODS

A retrospective analysis of publicly available surveillance data for influenza and RSV infections in the UK and Ireland from October 2020 to July 2021 as reported by Public Health England (PHE) and May 2021 as reported by Health Protection Surveillance Centre (HPSC). (6, 9-11)

RESULTS

Influenza and RSV activity during the 2020/21 winter season was considerably lower than in previous seasons. (6, 9-11)

Influenza

Influenza was not circulating during the 2020/21 season with very low activity levels in the UK and Ireland. (6, 9-11) General Practice (GP) influenza-like illness (ILI) consultation rates remained below baseline. (6, 9-11) In England, influenza hospitalisation and intensive care unit (ICU) admission rates remained below baseline for the duration of the season. (6, 9-11)

RSV

Reports of laboratory-confirmed RSV through DataMart and the National Virus Reference Laboratory (NVRL) were much lower than previous seasons in the UK and Ireland and the data suggest that the typical RSV season did not occur in winter 2020/21. (6, 9-11) In England, RSV positivity was ≤0.4% compared with peaks of 13.4% in 2019/20. Correspondingly, hospital admissions did not exceed 0.04/100,000 compared to peak weekly rates of 5.27/100,000 in 2019/20. (6) In Ireland as of 23rd May 2021, there were just 9 RSV notifications during the 2020/2021 season compared to 3,627 during the 2018/19 season. (7, 10)

Unseasonal RSV activity has recently been reported in England with RSV positivity increasing from 0.1% in May 2021 to 3.4% in July 2021. (8)

CONCLUSIONS

The results of this analysis confirm that seasonal influenza and RSV activity in the UK and Ireland was at unprecedentedly low levels during winter 2020/21. The impact of non-pharmaceutical COVID-19 mitigation measures on circulation patterns in the upcoming season is currently unknown. As COVID-19 restrictions are eased, we need to be prepared for a possible resurgence of influenza and RSV that may not follow the usual seasonal patterns.

The Academy of Medical Sciences warn that following an easing of COVID-19 restrictions and a loss of population immunity to some of the pathogens capable of causing RTIs, outbreaks of influenza and RSV during winter 2021/22 could be 1.5 to 2 times the magnitude of a normal year. (12)

Disclosure All authors are employees of Sanofi-Pasteur, UK and Ireland and has been developed and funded by Sanofi Pasteur, UK and Ireland.

References

Tang JW, Bialasiewicz S, Dwyer DE, Dilcher M, Tellier R, Taylor J, et al. Where have all the viruses gone? Disappearance of seasonal respiratory viruses in the UK and Ireland and the data suggest that the typical RSV season did not occur in winter 2020/21. (6, 9-11) In England, RSV positivity was ≤0.4% compared with peaks of 13.4% in 2019/20. Correspondingly, hospital admissions did not exceed 0.04/100,000 compared to peak weekly rates of 5.27/100,000 in 2019/20. (6) In Ireland as of 23rd May 2021, there were just 9 RSV notifications during the 2020/2021 season compared to 3,627 during the 2018/19 season. (7, 10)

Unseasonal RSV activity has recently been reported in England with RSV positivity increasing from 0.1% in May 2021 to 3.4% in July 2021. (8)


No paediatric RSV epidemic in 2020-2021 winter season: the upshot of COVID-19 pandemic

Topic: Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Laura Pellegrinelli (1), Cristina Galli (1), Laura Bubba (1), Arlinda Seiti (1), Giovanni Anselmi (1), Valeria Primache (2), Lucia Crostogini (2), Maria Gramegna (2), Sandro Binda (1), Elena Pariani (1)

1: Department of Biomedical Sciences for Health, University of Milan, Milan, Italy; 2: DG Welfare, UO Prevenzione, Lombardy Region, Milan, Italy

BACKGROUND
Before COVID-19 pandemic, RSV caused considerable paediatric morbidity, particularly in children under 5 years of age, resulting in a wide community transmission and more than 3 million of RSV-related hospitalizations every year. Since COVID-19 upsurge, the epidemiological pattern of respiratory pathogens has been overturned. In this study, we evaluated the community transmission of RSV in Lombardy (northern Italy) during the 2020-2021 winter season by detecting RSV circulation in the framework of influenza-like illness (ILI) surveillance and comparing it with surveillance data of the 5 previous seasons (from 2014-2015 to 2019-2020).

METHODS
In the framework of the Italian sentinel influenza surveillance network (InNet), nasal-pharyngeal swabs (NPS) were collected from paediatric ILI outpatients (<15 years) in the Lombardy region from 2014-2015 to 2020-2021 seasons (from week 46 to week 17 of the following year). NPSs were tested for RSV with specific real-time RT-PCR assay targeting the viral matrix gene.

RESULTS
From 2014-2015 to 2019-2020 season, 657 NPSs (median: 86 NPS/season; season range: 77-232 NPS) were collected from as many ILI cases <15 years (median age: 6 years, inter-quartile range [IQR]: 7 years). RSV was identified in 21.6% (142/657; annual range: 12.9-31.2%; weeks range: 6.1-37.2%) of NPS collected from children <15 years and in 30.9% (103/333; annual range: 16.9-39.6%; weeks range: 5.6-40%) of those from children <5 years. The risk of infection from RSV in children <5 years was nearly 22 times higher (OR: 22.8; 95%CI: 25.4-34.5) than that observed in the 5-15 years' age-group. Among ILI cases <5 years of age with underlying medical conditions/comorbidities, the risk of infection from RSV was 2.36 (95%CI: 2.36-9.03) higher than that observed in ILI cases <5 years of age. The average length of RSV epidemic was 11 weeks (range: 5-15 weeks) with RSV epidemic peak (week with the highest proportion of RSV-positive NPSs) between week 52 and week 10. In 2020-2021 season, 93 NPSs were collected from ILI cases <15 years (median age: 8 years, IQR: 10 years), none of them tested positive to RSV.

CONCLUSIONS
Since March 2020, non-pharmacological interventions (NIPs) (such as physical distancing, mandatory mask wearing, workplaces/schools closures) were massively implemented to contrast SARS-CoV-2 spread in Italy. This also affected RSV circulation that stopped circulating nearly 10 weeks earlier than during the 5 previous seasons and bringing to no RSV epidemic at all in 2020-2021 season. ILI surveillance was able to capture the upshot of COVID-19 pandemic in changing the community transmission of RSV; this may result in larger cohorts of young children naïve to RSV in the next years, in unexpected epidemiological pattern of RSV infection, and a new challenge in surveillance and RSV epidemic preparedness after NIPs relaxing.
The mutual impact of seasonal influenza and COVID-19 surveillance and Vaccine Coverage Rates (VCR) monitoring in Czech Republic, Greece, Poland, Romania and Slovakia

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Théophile Baisas (1), Joanna Chorostowska-Wynimko (2), Anca Drăgănescu (3), Claudiu El Guerche Sébètain (4), Zuzana Krstíková (5), Jan Kynel (6), Alireza Maoh (4), Mirela Puchýlita (7), Thierry Rigoine de Fougerolles (8), Georgios Saroglou (9), Olivier Vitoux (8)

1: CVA, London, United Kingdom; 2: National Institute of Tuberculosis and Lung Diseases, Warsaw, Poland; 3: National Institute for Infectious Diseases “Prof. Dr. Matei Balș”, Bucharest, Romania; 4: Sanofi Pasteur Lyon, France; 5: Public Health Faculty, Slovak Medical University, Bratislava, Slovakia; 6: Department of Infectious Diseases Epidemiology, National Institute of Public Health, Prague, Czech Republic; 7: Sanofi Pasteur Bucharest, Romania; 8: CVA, Paris, France; 9: National and Kapodistrian University of Athens, Athens, Greece

**BACKGROUND**

Public health surveillance systems in Czech Republic, Greece, Poland, Romania and Slovakia routinely collect epidemiological data on influenza from sentinel sites in primary and secondary care for which biological samples are analysed by reference laboratories. In response to the coronavirus disease 2019 (COVID-19) pandemic, the World Health Organisation (WHO) issued guidance on adapting national surveillance systems to monitor Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) viruses while maintaining influenza surveillance. This study aims to describe the extent to which influenza surveillance systems and VCR monitoring systems were repurposed, enhanced or replaced for COVID-19, and how this could in turn impact influenza surveillance.

**METHODS**

The study utilised a previously developed framework for the systematic comparison of influenza & COVID-19 surveillance systems, structured around 7 sub-systems and 20 outcomes of interest and relying on 5 evaluation criteria inspired from WHO guidance. VCR monitoring was added as an additional sub-system including outcomes for each vaccination target group. Details on the outcomes collected and communicated to the public as part of the surveillance and VCR monitoring for influenza and COVID-19 were gathered from official epidemiological reports, vaccination campaign reports and other publicly available resources from European and national public health agencies. The results were reviewed and discussed with a panel of 10 surveillance experts from Czech Republic, Greece, Poland, Romania and Slovakia.

**RESULTS**

The COVID-19 pandemic fostered the introduction of several new surveillance tools and a shift to mandatory notification of laboratory-confirmed cases, hospitalizations and deaths in all five countries. Only a limited number of sub-systems used for influenza surveillance such as reference virological laboratories and death registers were leveraged. Serological surveys, monitoring of outbreaks in public closed settings and extended hospital surveillance were established for COVID-19. Systematic case reporting and widespread testing led to more granular, representative and accurate data whilst communication practices also improved through the use of interactive dashboards and maps. COVID-19 VCR monitoring systems experienced a step change improvement when compared to influenza as Czech Republic, Greece, Poland, Romania and Slovakia introduced dedicated electronic registries. While improvements were made with regards to geographic and age stratification, VCR data per risk group such as amongst pregnant women often remain unavailable as for influenza.

**CONCLUSIONS**

While the pandemic funnelled significant resources into new surveillance tools for COVID-19, their applicability for seasonal influenza remains to be determined. Furthermore, approaches such as influenza-like illness sentinel schemes face durable disruption due to the emergence of this new pathogen with overlapping symptoms, raising the question of how and to what extent they will be sustained by health authorities in coming years. For VCR monitoring, COVID-19 electronic vaccination registries will be reused for influenza in Greece, but their repurposing remains unclear in the other countries.

**Funding** This work was funded by Sanofi Pasteur

**Keywords** Central and Eastern Europe; framework; influenza; COVID-19; surveillance; vaccine uptake; vaccine coverage rates
The mutual impact of seasonal influenza and COVID-19 surveillance and Vaccination Coverage Rates (VCR) monitoring in Belgium, Israel, Norway, Sweden and the Netherlands

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Théophile Baissas (1), Clotilde El Guerche Sébèan (2), Kristin Krogh (3), Alireza Mafi (2), Michal Mandelboim (4), John Paget (5), Thierry Rigone de Fagerolles (6), Bas Slierendregt (7), Lena Svensson (8), Marc Van Ranst (9), Linda Vercammen (10), Olivier Vitéaux (6)

1. CVA, London, United Kingdom;
2. Sanofi Pasteur, Lyon, France;
3. Sanofi Pasteur, Oslo, Norway;
4. Central Virology Laboratory, Ministry of Health, Chaim Sheba Medical Center, Tel Hashomer, Ramat Gan, Israel;
5. Netherlands Institute for Health Services Research, Utrecht, Netherlands;
6. CVA, Paris, France;
7. Sanofi Pasteur, Amsterdam, Netherlands;
8. Sanofi Pasteur, Stockholm, Sweden;
10. Sanofi Pasteur, Diegem, Belgium

**BACKGROUND**

Seasonal influenza surveillance systems have long been established in Belgium, Israel, Norway, Sweden and the Netherlands, leveraging tools ranging from laboratory networks to sentinel schemes and statistical modelling designed to inform public health decisions on prevention against influenza epidemics. In response to the coronavirus 2019 (COVID-19) pandemic, the World Health Organisation (WHO) issued guidance on adapting national surveillance systems to monitor SARS-CoV-2 viruses while maintaining influenza surveillance. This study aims to describe the extent to which influenza surveillance systems and VCR monitoring systems were repurposed, enhanced or replaced for COVID-19, and how this could in turn impact influenza surveillance.

**METHODS**

The study utilised a previously developed framework for the systematic comparison of influenza & COVID-19 surveillance systems, structured around 7 sub-systems and 20 outcomes of interest and relying on 5 evaluation criteria inspired from WHO guidance. VCR monitoring was added as an additional sub-system including outcomes for each vaccination target group. Details on the outcomes collected and communicated to the public as part of the surveillance and VCR monitoring for influenza and COVID-19 were gathered from official epidemiological reports, vaccination campaign reports and other publicly available resources from European and national public health agencies. The results were reviewed and discussed with a panel of 12 surveillance experts from Belgium, Israel, Norway, Sweden and the Netherlands.

**RESULTS**

Health authorities repurposed influenza surveillance tools such as sentinel networks and excess-mortality modelling, leveraging established surveillance systems. New tools were introduced for COVID-19 spanning hospital capacity and intensive care registers, mass serological testing in the community, as well as self-reported community surveys and case notification in nursing homes. The stratification of data improved considerably across age, time and space as did its representativeness and accuracy given the unprecedented use of molecular and antigenic testing. Monitoring of COVID-19 VCR was established using national registries rather than less representative methods used for influenza, thus achieving a uniformity of data across regions and reporting from all recipients.

**CONCLUSION**

Capitalizing on existing channels and databases for influenza surveillance and VCR monitoring proved valuable for pandemic preparedness and COVID-19 vaccination roll-out in the five countries considered. In the unprecedented COVID-19 context, the scope of data collection was expanded to new indicators measuring the broader pandemic impact on the healthcare systems and public closed settings, or the vaccination status amongst healthcare workers and nursing home residents. Nonetheless outcomes such as pregnancy VCR data remain missing for both vaccines. Overall, data communication was considerably improved through digital automation and almost real-time data flows. While disruptions to influenza sentinel systems and mortality modelling could occur due to the pandemic impact, developments observed in the context of COVID-19 provide opportunities to level the surveillance of influenza.

**Funding** This work was funded by Sanofi Pasteur.

**Keywords** Northern Europe, Israel, framework; influenza, COVID-19; surveillance; vaccine uptake; vaccine coverage rates;
Low number of influenza hospitalisations in Norway 2020-2021

Topic: Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Ragnhild Tønnessen (1,3). Mari Grøgaard (2), Ketil Telle (2), Jesper Dahl (1), Torstein Aune (1), Robert Neil Whittaker (1), Trine Hessevik Poulsen (1)

1: Division of Infection Control and Environmental Health, Norwegian Institute of Public Health, Oslo, Norway;
2: Division for Health Services, Norwegian Institute of Public Health, Oslo, Norway;
3: European Public Health Microbiology Training Programme (EUPHEM), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

BACKGROUND

Since the upsurge of the COVID-19 epidemic in 2020, a concern for an influenza and COVID-19 “twindemic” that could overburden hospital capacity has been raised. To enhance influenza surveillance in Norway, a national register-based surveillance system for influenza hospitalisations was established. We describe the number of influenza hospitalisations in Norway during 2017-2021, in order to assess the severity of the 2020-2021 influenza season during the COVID-19 epidemic.

METHODS

Individual-level hospital surveillance data came from The Norwegian Patient Registry (NPR). Influenza hospitalisations were defined as inpatient hospital admissions combined with codes from the International Classification of Diseases, tenth revision (ICD-10) for influenza (J09-J11). The data for each season (week 40 to week 20) were stratified per sex and age. Only the first admission with influenza per season was included.

RESULTS

In total 25 cases of influenza hospitalisations (J09-J11) were registered in Norway during the 2020-2021 season, compared to an average of 4979 hospitalisations per season (range 2814-7497) for the seasons 2017-2018 to 2019-2020 (Figure 1). The median age of the cases in 2020-2021 was 48 years and 64% were male, compared to a median age of 62 years and 50% males in the three preceding seasons.

CONCLUSIONS

The severity of the 2020-2021 season was extremely low, which reflects the impact that measures to reduce the spread of COVID-19 probably had on transmission of influenza virus. The absence of influenza may have reduced population immunity, which emphasises the importance of vaccination to prevent severe disease and death in risk groups and close monitoring of both influenza and COVID-19 hospitalisations during the upcoming season, especially as COVID-19 restrictions are lifted.

Figure 1. Weekly number of hospitalised cases with influenza (J09-J11) in Norway by season 2017-2021.
Sentinel Surveillance of Severe Acute Respiratory Infections in Serbia during the COVID-19 pandemic

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Dragana Dimitrijevic, Verica Jovanovic
Institute of Public Health of Serbia, Serbia

**BACKGROUND**
Sentinel surveillance of severe acute respiratory infections (SARI) was implemented in Serbia in November 2009. 10 sentinel hospitals in 4 cities participated in the SARI surveillance system.

Infectious disease, pediatric Intensive care units (ICUs), and respiratory disease wards were all represented. The aim of this study is to provide a review of sentinel surveillance of SARI in Serbia during COVID 19 pandemic.

**METHODS**
During October 2019 to August 2021, both epidemiological and virological influenza data were collected and analyzed on a national level and weekly basis. Data are reported at the national level to ECDC and the WHO Regional Office for Europe through The European Surveillance System (TESSy) on a weekly basis during the influenza surveillance season. For laboratory confirmation of influenza, Real timepolymerase chain reaction (RT-PCR) was used.

**RESULTS**
In the Republic of Serbia, the first case of COVID-19 was reported on 6 March 2020, and the outbreak is still ongoing. Start of influenza in 2019/2020 season was registered in week 50/2019.

Influenza activity peaked between weeks 3/2020 and 7/2020, with the positivity rate higher than the >50%. A total of 989 SARI cases were reported. Among these cases, 572 (57.8%) respiratory specimens were collected and tested. The number of positive samples was 221 (38.6%). The highest proportion of laboratory-confirmed influenza cases was 60% in week 3/2020. Both influenza type A (A/HL/ pdm09 and A (H3)) and type B viruses were detected.

There were no hospitalized laboratory-confirmed influenza cases during 2020/2021 influenza season. In total, 10 deaths among sentinel SARI laboratory-confirmed influenza cases have been reported. All of them were from intensive care units, ICUs.

**CONCLUSIONS**
Seasonal influenza activity was lower in season 2020/2021 than in previous years in Serbia without laboratory confirmation of influenza. Influenza activity may have been affected by measures taken to constrain the SARS-CoV-2 outbreak.

Integration of laboratory surveillance with epidemiological surveillance for both pathogens, influenza and SARS-CoV-2, in the lights of Covid 19 pandemic is priority in Serbia and rises the core surveillance capacity.
Impact of COVID-19 outbreaks on influenza in Montenegro during flu season 2020-2021

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

**Bozidarka Rakocevic (1), Sanja Medenica (1), Karolina Glavatovic (1), Ivan Lekic (1), Olivera Bojovic (2), Boban Mugosa (1)**

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**BACKGROUND**

Coronavirus disease 2019 (COVID-19) is a pandemic influencing the first half of the year 2020. SARS-CoV-2 has rapidly spread to many countries and extensive public health and societal measures have been implemented to slow virus transmission. This study was conducted to describe epidemiological situation of COVID-19 and influenza-like illness (ILI) in Montenegro during flu season 2020-2021.

**METHODS AND MATERIALS**

Influenza surveillance is implemented annually during the influenza season from week 40 to week 20. Weekly data of ILI cases and laboratory confirmed cases of flu and COVID-19 for the referring period were obtained from the Institute of Public Health of Montenegro. World Health Organisation’s definition of ILI case and COVID-19 case were used. Every COVID-19 case have been sampled with respiratory swab tested for SARS-CoV-2 by polymerase chain reaction or antigen test. Descriptive epidemiological method was used for this study.

**RESULTS**

During observed period baseline activity of influenza was registered, without any laboratory confirmed case of flu. There were 87388 laboratory confirmed COVID-19 cases, of which 48.47% % in men. The incidence in the observed period were ranged from 63.38/100 000 inhabitants in week 20 to 638/100 000 inhabitants in week 10 (Fig 1).

![ILI incidence and COVID-19 incidence during flu season 2020-2021](image)

*Fig 1. ILI incidence and COVID-19 incidence during flu season 2020-2021*

In the observed period, 1428 people died and the case fatality rate was 1.6%. 923 cases (64.63%) were men. 77,39 % were older than 64 years.

**CONCLUSIONS**

The ongoing COVID-19 pandemic has resulted in implementation of public health measures worldwide to mitigate disease spread, including: travel restrictions, lockdowns, instructions on handwashing, use of face coverings and physical distancing and had impact to spread of flu infection.

*Keywords*: COVID-19, ILI, Montenegro;
Sentinel surveillance using free ranged domestic birds for Avian influenza virus in wild birds

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Erdene-Ochir Tsiren-Ochir (1), Bayarmagnai Davganyam (1), Narandasaar Batsuuri (1), Sun-Hak Lee (2), Nyamsuren Otgonlogtok (1), Jeong Sol (2), Nyamdavaa Guugandaa (1), Tungalag Chultemdorj (1), Chang-Seon Song (2)

1: Mongolian University of Life Sciences, Mongolia; 2: Konkuk University, Seoul, South Korea

**BACKGROUND**

Wild birds have been implicated in the spread of HPAI of the H5Nx subtype, prompting surveillance among wild birds. Although we implemented surveillance for AIV using fresh fecal sampling method, but its isolation rate has lower than swab sampling methods for captured or hunted birds that prohibited in Mongolia. Thus, it’s important to develop an effective method for avian influenza surveillance.

**METHODS & MATERIALS**

In August–October, 2020, active surveillance for avian influenza virus using sentinel ducks were conducted in Mongolia (Khunt lake, Bulgan province) that major wild bird habitat and outbreak site of HSN1 HPAI in wild birds in Mongolia from 2005 to 2011 and H5N6 HPAI in 2020.

Domestic ducks (n=10) were free ranged in small island of lake. Cloacal and tracheal swabs and serum samples were collected a weekly and viral RNA extracted using Viral Gene-spin™ Viral DNA/RNA Extraction Kit and analyzed by real time PCR (qPCR) and serums were tested by AIV NP Ab ELISA kit. In addition, domestic ducks (n = 5) were placed animal facility of university for negative control group.

**RESULTS**

Total of 9/10 (90%) birds were AIV positive by qPCR and two subtype LPAIVs isolated including H3 (n=8) and H4 (n=1). In addition, AIV antibody detected by AIV NP ELISA kit in 10/10 (100%) birds. A negative control group were negative by all tests.

**CONCLUSION**

The detection rate of AIV RNA (90%) and Ab (100%) using free ranged sentinel domestic ducks was higher than previous experiment that sentinel domestic ducks were placed in cage. These results indicated that sentinel surveillance using free ranged domestic birds could be an effective method for avian pathogens including influenza in Mongolia. Enhanced sentinel surveillance in wild bird populations in Mongolia is therefore crucial for the understanding of global AIV transmission and epidemiology.
Epidemiology of the first wave of the COVID-19 epidemic in Montenegro

Topic: Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Sanja Medenica, Adriana Vujovic, Bozidara Rakocic, Zorica Djordjevic, Boban Mugosa
Institute of Public Health of Montenegro, Podgorica, Montenegro

INTRODUCTION
The family Coronaviridae consists of a wide range of animal and human viruses, with a characteristic morphology. These are enveloped, spherical, or disk-shaped RNA viruses.

OBJECTIVE
To investigate the epidemiological characteristics of the first wave of the COVID-19 epidemic in Montenegro.

MATERIAL AND METHOD
A descriptive epidemiological method was used in this paper. The data source is an electronic database for reporting SARS-CoV-2 positive microbiological results and an epidemiological survey.

RESULTS
In the period from March 17 to June 2, 2020, 324 people in Montenegro fell ill with COVID-19, which is an incidence of 52.25 / 100,000 inhabitants. In the observed period, 74 people were hospitalized from COVID 19. The total number of deaths in the observed period is 9, which represents a mortality rate of 1.45 / 100,000 and a fatality rate of 2.7%. The last case of the disease was registered on May 4. The largest number of patients was registered in the age group of the working population aged 20-60. The incidence of men was higher in relation to women, 55.8% men and 44.2% women. The youngest patient was one year old, and the oldest was 85 years old. The average age structure of patients is 43 years.

CONCLUSION
A multisectoral approach to controlling epidemic outbreaks yields the best results. The first battle was successfully completed thanks to the conscientious and responsible behavior of every inhabitant of Montenegro.

Fig. 1. The number of cumulative cases of COVID-19, in period 17.03.-04.05.2020, in Montenegro

Keywords COVID-19, SARS-CoV-2, Montenegro
Public Health Policies quantitative modelling towards increased influenza vaccination coverage rates

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Henrique Lopes (1), Rifat Atun (2)

1: Institute of Health Sciences, Universidade Católica Portuguesa (Catholic University of Portugal), Lisbon, Portugal; 2: Department of Global Health and Population, Harvard TH Chan School of Public Health, Harvard University, Boston, MA, USA

**BACKGROUND**

The management of infectious diseases greatly relies on the design and timely implementation of evidence-based health policies as revealed by the current pandemic. Effective health policies are needed to ensure appropriate global preparedness for influenza worldwide. While the public health measures put in place to fight COVID-19 have led to sharp declines in the number of flu cases and deaths, there is still a risk of a confluence of COVID-19 and flu pandemics, which must be avoided. Fake news against scientific evidence, especially vaccination, creates misinformation and fear, which hinder vaccination coverage rates (VCR).

Influenza vaccination is acknowledged as the best tool to control the disease if supported by appropriate health policies to develop effective influenza vaccination campaigns to maximize vaccination coverage and uptake.

**METHODS**

This study aims to develop a mathematical model of health policies for influenza vaccination in a project entitled “Let’s Control Flu” (LCF)—an international collaboration that began in 2021. The LCF project includes the development of a gamified interactive tool integrated with analytical algorithms to assess the potential effects and impact of health policies aimed at expanding coverage and uptake of flu vaccination, using a quantitative approach to a qualitative conceptual model developed by Kassianos (2021). The development of the model tool is underway, with its application planned for five countries, starting with Sweden.

**RESULTS**

The LCF tool allows countries to easily access different health policy scenarios directed at optimizing VCR. This enables different social actors with varied knowledge of influenza to engage in the policy option generation process, strengthening health democracy. Also, it helps raise awareness on influenza, policies and actions aimed at preventing the disease. The interactive tool enables the engagement of stakeholders interested in the control of a potential flu epidemic, from patient advocates to health authorities and political actors, in the identification of an appropriate set of health policies aimed at expanding VCR to achieve epidemiological impact. Modelled scenarios enable a real-time view of how different mix of health policies may impact influenza control outcomes (Campaign Accountability; Access to Vaccination; HCP Engagement; Burden of Disease Awareness; Communication with the Public) and outputs (Infection cases; GP visits; Hospitalization; Workday loss; Deaths; Influenza-related Cardiovascular events). The modelling focuses on key target groups for the influenza vaccination policies: Children; Healthcare and social workers; Pregnant women; Elderly; High-risk individuals.

**CONCLUSIONS**

The quantitative modelling of the potential effects and impact of health policies on influenza enables the development of an appropriate policy mix and sequencing to optimize VCR. The easy-to-use tool allows the engagement of multiple stakeholders in considering policy options and helps foster greater awareness for interventions aimed at the prevention of the disease to help reduce infection, transmission, ill-health and death.

**Keywords:** Influenza, Vaccination, Modelling, Public Health Policies
Identification of amino acid substitutions involved in the zoonotic transmission of the 2009 pandemic H1N1 using phylogenetic and ancestral inference analysis

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Kiara Marie Andersen (1, 2), Jakob Nybo Nissen (1), Anders Gorm Pedersen (2), Ramona Trebbien (1)

1: Statens Serum Institut, Denmark
2: Technical University of Denmark

**BACKGROUND**

The zoonotic potential of Influenza A viruses (IAVs) poses an obvious threat of emerging human pandemics. The latest IAV pandemic in 2009 is thought to originate from Mexican swine and was a reassortment between three different swine IAV (swIAV) lineages. However, sequencing data of swIAVs from before 2009 is scarce, and thus we have no clear interpretation of the mutational events that lead to this zoonotic transmission.

**METHODS**

In the search for genetic markers of zoonotic IAVs to be used in future surveillance, we used extensive phylogenetic analyses of each viral gene segment to map the genetic events leading to the 2009 pandemic H1N1 (H1N1pdm09). We collected all available sequence data from each segment of swIAVs from public databases (NCBI Influenza Virus Resource and GISAID) within a timespan of 5 years before and after the pandemic emergence. Additionally, we collected swIAV sequences found by BLAST searches to the human 2009 pandemic reference strain, A/California/07/2009. Phylogenetic analyses using both BioNJ and maximum likelihood approaches then allowed us to infer ancestral sequences to the H1N1pdm09 subtype and to identify mutations possibly related to the zoonotic transmission.

**RESULTS**

We have reconstructed ancestral sequences to H1N1pdm09 and identified potentially important amino acid substitutions in each viral protein along the evolutionary branches preceding H1N1pdm09. An example of the analysis for the PB2 gene is shown in Figure 1. Only a few of the identified substitutions have already been described in literature in relation to e.g. host adaptation, however, most of the substitutions still need further characterization.

**CONCLUSIONS**

Additional and more extensive analyses of the amino acid variations in each segment, both individually and in combination with each other, should be conducted to reveal their impact on the emergence of the zoonotic event. This knowledge is valuable in the surveillance of circulating IAVs in human and swine and should be used to evaluate the zoonotic potential of emerging viruses for prevention of future pandemics.

**References**


![Figure 1: Phylogenetic tree of the PB2 gene and amino acid substitutions between ancestors to the H1N1pdm09 clade. All sequences are from IAVs of swine origin, except A/California/07/2009, which is highlighted in green. Descendants of H1N1pdm09 in swine are collapsed and the ancestral branches leading to the H1N1pdm09 clade are colored in green. Inferred amino acid substitutions are shown on top of the branches where they occurred. Substitutions previously described in literature to have possible connections to host adaptation are shown in blue.](Image)
Comparison of amplicon-based conventional and long-read Illumina sequencing for high-resolution variant profiling of H3N2 Influenza A virus HA recovered from a clinical sample

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Temitepe Faleye (1), Deborah Adams (2), Sangeet Adhikari (1,3), Helen Sandrolini (4), Rolf Halden (1,3,5), Arvind Varsani (6), Matthew Scotch (1,7)

1. Biodesign Center for Environmental Health Engineering, Biodesign Institute, Arizona State University, Tempe, AZ, USA 85287; 2. Biodesign Center for Personalized Diagnostics, Biodesign Institute, Arizona State University, Tempe, AZ, USA 85287; 3. School of Sustainable Engineering and the Built Environment, Arizona State University, Tempe, AZ, USA 85287; 4. Arizona State University Health Services, Arizona State University, Tempe, AZ, USA 85287; 5. OneWaterOneHealth, The Arizona State University Foundation, The Biodesign Institute, Arizona State University, Tempe AZ 85281; 6. Biodesign Center for Fundamental and Applied Microbiomics, Center for Evolution and Medicine, School of Life Sciences, Arizona State University, Tempe, AZ, USA 85287; 7. College of Health Solutions, Arizona State University, Phoenix, AZ, USA 85004

**BACKGROUND**

Influenza virus (IV) variant profiling from clinical samples currently leans largely on amplicon-based conventional Illumina sequencing (cIS) and single nucleotide variant (SNV) analysis. This is sometimes coupled with probabilistic predictions to determine co-evolving sites within each genomic segment. Here we describe the use of long-read Illumina sequencing (LRIS) for characterizing Influenza A virus (IAV) HA contig diversity in a clinical sample and compare the use of amplicon-based cIS and LRIS for variant profiling.

**METHODS**

We used an H3N2 IAV from a respiratory swab collected in December 2019 as part of our annual IV surveillance study in a university community in Tempe, Arizona, USA. The sample was subjected to RNA extraction, cDNA synthesis, complete segment PCR, cIS and LRIS. The raw reads were quality checked and assembled using Spades. The cIS generated contigs were analyzed for SNVs while the LRIS contigs were analyzed for variant contigs and co-evolving sites.

**RESULTS**

Using cIS, one HA contig with no SNVs was recovered (coverage 30,404x). Using LRIS, 66 contigs were recovered (total coverage 11,947x). Of the 66 HA contigs, one had 42 copies, four had 2 copies each and the remaining 16 had single copies (singletons). Hence, 21 unique contigs were recovered by LRIS. The contig with 42 copies (total coverage 7,570x) had exactly the same sequence as the consensus recovered using cIS (coverage 30,404x). When in-silico translated, 4 of the singleton contigs had the same amino acid (AA) sequence as the contig with 42 copies. Hence, viewed by AA sequence, one and 18 unique HA-H3 sequences were recovered by cIS and LRIS, respectively. Compared to the dominant AA sequence, 12 had one AA substitution each, three had 2 AA substitutions each, while the remaining two had 3 (coverage 171x) and 4 (coverage 210x) AA substitutions each.

**CONCLUSION**

In this study, we describe the use of amplicon-based LRIS to delineate at high resolution, HA variants directly from a H3N2 containing nasopharyngeal swab sample. The workflow we describe makes it possible to monitor, not just variant sites in a genomic segment, but variant contigs as a unit and as a consequence, co-evolving sites without the need for probabilistic predictions. The method described here will also make it possible to study at higher resolution within-host evolution of antigenic variants and antiviral resistance alongside how these play into IV global transmission dynamics and evolution.
Global Estimates of Influenza-associated Respiratory Hospitalization from the Influenza Burden, Global Project (iCEBERG)

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Danielle Iuliano (1), Howard Chang (1), Neha Patel (1,3), Vanessa Cozza (4), Ben Cowling (5), David Muscalu (6), Cheryl Cohen (7), Verran Lee (8,9), Stefana Tempia (1), Richard Pebody (10), Melissa Rolles (1), Katie Lafond (1), Shikha Garg (1), Katherine Roguski (1), Eduarda Aziz-Baumgartner (1), Carrie Reed (1), Julia Fitzner (4), For The iCEBERG Collaborator Network (1)

1. Centers for Disease Control & Prevention, United States of America;  
2. Department of Biostatistics and Biomathematics, Rollins School of Public Health, Emory University, Atlanta, GA, USA;  
3. Chickasaw Nation Industries, Inc., Norman, Oklahoma, USA;  
5. WHO Collaborating Centre for Infectious Disease Epidemiology and Control, School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR;  
6. University of New South Wales, New South Wales, Australia;  
7. Centre for Respiratory Diseases and Meningitis, National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa;  
8. Ministry of Health, Singapore;  
9. Saw Swee Hock School of Public Health, National University of Singapore, Singapore;  

**BACKGROUND**
Seasonal influenza epidemics are an important contributor to the annual global hospitalization burden. Understanding hospitalization burden in countries can provide information about the severity of seasonal epidemics, support policy decisions regarding prevention and control measures, and to assist in the identification of unusual or increased activity. However, many countries have insufficient data to estimate influenza hospitalization burden in their populations. We propose a method to estimate influenza-associated hospitalizations for 185 countries.

**METHODS**
We evaluated influenza surveillance systems, hospitalization databases, and rate calculation methods from countries with influenza-associated hospitalization estimates for contribution to a global extrapolation model. We used hospitalization rates from 2007-2018, four age groups (<5, 5-49, 50-64, and ≥65 years), and identified country-specific covariates (e.g. economic, geographic, healthcare systems, healthcare access) to adjust for differences in hospitalization risk between countries. Bayesian hierarchical models were used to estimate associations between age-specific influenza-associated hospitalization rates and country-specific covariates accounting for uncertainty in rate estimates and covariate selection. Predictive inference from these models was used to extrapolate hospitalization rates to countries without rates.

**RESULTS**
As of 28 Apr 2020, 56 collaborators shared national or regional influenza-associated hospitalization rates. The highest country-specific influenza-associated hospitalization rates were among children <5 years (mean: 207.9/100,000 population), followed by adults ≥65 years (mean: 197.9/100,000). After extrapolation to 185 countries, we estimated 3,793,400 (4,564,000-9,702,000) global influenza-associated hospitalizations (rate: 76.1/100,000) occur annually. The highest median influenza-associated hospitalization rate was among those aged <5 years (287.5/100,000), followed by those ≥65 years (212.9/100,000). High income countries (141.8/100,000) had higher influenza-associated hospitalization rates compared with lower-middle income countries (45.9/100,000).

**CONCLUSIONS**
Globally, influenza infections are associated with >5,793,000 hospitalizations annually. Young children, older adults, and individuals in high-income settings were at disproportionately greater risk of hospitalization compared with other age groups and income classifications. These differences may be due to health seeking behaviors in populations and availability of or access to healthcare services. Hospitalization estimates may inform about the burden of influenza infections on the population and healthcare settings and guide interventions.
Unusual timing of RSV circulation in the SARS-CoV-2 pandemic period in Slovenia

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

*Katarina Prosenc Trilar (1), Natasa Berginc (1), Maja Sočan (2)*

1: National Laboratory for Health Environment and Food, Slovenia; 2: National Institute of Public Health, Slovenia

**BACKGROUND**

Onset and offset of respiratory syncytial virus (RSV) epidemic vary from season to season with median start in Europe being week 49 and in Slovenia week 50–51. In temperate climate annual patterns of RSV circulation are strongly associated with meteorological conditions, the temperature and atmospheric pressure being the main factors. In season 2020/21 with pandemic of SARS-CoV-2, in Slovenia an unusual pattern of RSV circulation was observed.

**METHODS**

In Slovenia, the RSV surveillance was established in 2006 and is based on the national laboratory data. All laboratories are reporting on weekly basis the number of patients tested for RSV and the of positive and negative results. Mostly samples are taken for diagnostic purposes in physician sole discretion. Samples are tested with real-time multiplex PCR including detection of RSV and other respiratory viruses.

The data from five previous seasons were compared with data from 2020/21 season and association of RSV circulation with implementation of non-pharmaceutical interventions (NPI) for mitigation of SARS-CoV-2 pandemic, were studied.

**RESULTS**

In seasons 2015/16, 2016/17, 2017/18, 2018/19, 2019/20, there were 16,273, 18,291, 18,031, 18,832, 21,460 respiratory specimens tested for RSV, respectively. In the season 2020/21 up to week 34 19,760 specimen were checked for RSV. The 7% RSV positivity rate in seasons 2015/16 to 2019/20 was reached from week 51 to week 4 (Median 52), and dropped under 7% positivity from week 11 to 17 (Median 15). In the pandemic season 2020/21 no circulation of RSV or just sporadic cases were observed till week 23 and from then on steady rise was observed, reaching 7% positivity rate in week 32 and reached 10.6% in week 34 that was last week of study. NPI, namely closure of kindergartens, primary and secondary schools were in effect in periods from weeks 10/2020 to 22/2020, from week 47/2020 to 5/2021 and in weeks 13 and 14/2021. Some other NPI as restrictions on public and private gatherings, non-essential shops’ closure, workplace closure, stay at home order etc were partly or fully coinciding with closure of schools. Eastern part of the country has winter school vacation in week 7/2021 and Western in week 8/2021.

**CONCLUSIONS**

In season 2020/21 the autumn to spring circulation of RSV was missing in Slovenia as well as in many European countries, except in France and Iceland, but it was delayed and lasted for shorter period. In Slovenia incidence of RSV started to rise in late early July 2021. Release of lock-down NPIs and increased movement of people has accelerate circulation of RSV despite absence of meteorological factors usually conditioning the RSV epidemic. Development of this RSV epidemic is questionable an pose a challenge how to plan the timing of monoclonal antibodies application for risk group infants.
Phylogenetic analysis of reverse zoonotic influenza A subtype H3N2 virus in Danish swine

Topic: Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Jakob Nissen (1), Lars Larsen (2), Charlotte Hjulsager (1), Ramona Trebbien (1)

1: Statens Serum Institut, Denmark;
2: University of Copenhagen

BACKGROUND
Swine has long been considered an intermediate host for reassortment of influenza A virus with human pandemic potential. The latest influenza pandemic in 2009 was such a reassortant H1N1 swine influenza virus. The last decades has seen recurrent reverse zoonoses of human-adapted H3N2 in swine, however in most cases after H3N2 reverse zoonosis, the internal gene segments become replaced with H1N1 segments over time[1]. Despite approximately 10% of genotyped influenza virus in Danish swine contain human-derived segments[2], a full human-adapted H3N2 virus has never been seen in Danish swine.

METHODS
During routine surveillance of a Danish swine herd with severe clinical signs of influenza infection, nasal swab and blood samples were collected from the herd. Serum was tested in hemagglutinin inhibition assays and tested for the presence of influenza A antibodies. Influenza RNA was extracted from nasal swab samples and full-genome sequenced. Sequences were assembled and phylogenetically analyzed.

RESULTS
All influenza-positive samples contained virus descended from, and closely related to, human-adapted influenza A of clade 3C.3a, circulating in the Danish population in 2019-2020 season. Several mutations relative to the closest known human virus were identified.

CONCLUSIONS
Despite segments descendend from human seasonal H3N2 are common in Danish swine, this is the first time a complete virus of this type has been detected in swine in Denmark. The severe clinical symptoms imply the virus was capable of efficient replication in swine with either no or little adaptation.


Respiratory Pathogens Circulating Among Severe Acute Respiratory Syndrome Coronavirus-2 Infection Suspected Patients During The Ongoing Covid-19 Pandemic In Central Province Of Sri Lanka

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Shiyamalee Arunasalam (1), F. Naureen (1), R. Muthugala (2)

1: University of Peradeniya, Sri Lanka; 2: National Hospital, Kandy, Sri Lanka

**BACKGROUND**
The clinical symptoms of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infections are similar to other respiratory infections. Aetiological diagnosis during the COVID-19 pandemic is only focused on SARS-CoV-2 in laboratories in many countries. Thus knowing the epidemiological patterns of other respiratory pathogens is valuable to improve the diagnostic efficacy during the pandemic in COVID-19 suspected patients with acute respiratory tract infection (ARTI) symptoms especially in low / low middle income countries where diagnostic services are limited.

**METHODS**
In an ongoing prospective study, a total of 223 respiratory samples from COVID-19 suspected patients with symptoms of ARTI received to the Virology Laboratory of the National Hospital, Kandy, Sri Lanka were simultaneously tested using rtRT-PCR for SARS-CoV-2 and PCR melting curve analysis for 22 other respiratory pathogens from 1st of January to 30th of June 2021. The demographic and clinical data were acquired from medical records.

**RESULTS**
Only 6% (13/223) patients with suspected COVID-19 were eventually confirmed to have SARS-CoV-2 infection. Overall detection rate of other respiratory pathogens was 51% (113/223) including human rhino / enterovirus (22%), human coronavirus - 229E (13%), adeno virus (13%), RSV-A (10%), human parainfluenza virus-3 (10%), and human coronavirus NL-63/HKU 1 (10%). Overall positivity of other respiratory viruses was higher in males (70%) than in females (30%) and the age of the patients ranges from 14 days to 85 years. The time of sample collection for testing was less than 5 days from the onset of symptoms in 94% of patients and more than 5 days in 5% of patients. Twenty three respiratory viral co-infections were noted including six with SARS-CoV-2.

**CONCLUSION**
The current findings highlight the importance of diagnosing the other respiratory pathogens and their clinical impact during the ongoing COVID-19 pandemic. This can help support to initiate appropriate management plans while eliminating unnecessary patient isolation.

**Keywords** COVID-19, Respiratory pathogens, Epidemiology, Central Province, Sri Lanka
Clinical characteristics and population-based attack rates of respiratory syncytial virus versus influenza hospitalizations among adults

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Raija Auvinen (1,2,3), Ritva Syrjänen (4), Jukka Ollgren (2), Hanna Nahtynek (2), Kirsi Skagberg (1)

1: Inflammation Center, University of Helsinki and Helsinki University Hospital;
2: Infectious Disease Control and Vaccinations Unit, Department of Health Security, Finnish Institute for Health and Welfare;
3: Internal Medicine and Rehabilitation, University of Helsinki and Helsinki University Hospital;
4: Population Health Unit, Department of Public Health and Welfare, Finnish Institute for Health and Welfare, Helsinki, Finland

**BACKGROUND**
The clinical significance of respiratory syncytial virus (RSV) among adults remains under-investigated. We compared the characteristics and population-based attack rates of RSV and influenza hospitalizations.

**METHODS**
During two epidemic seasons 2018-20, we recruited all eligible community-dwelling hospitalized adults with severe acute respiratory infection (SARI) to our prospective study at a tertiary care hospital in Finland and compared the clinical characteristics of RSV and influenza patients. In our retrospective register-based study, we calculated the attack rates of all acute RSV and influenza hospitalizations in the same background adult population living in the hospital catchment area during four epidemic seasons 2016-20.

**RESULTS**
Of the 537 prospective study patients, 31 (6%) had RSV and 106 (20%) had influenza. Duration of hospitalization, need for intensive care or outcome did not differ significantly between RSV and influenza patients. RSV was more often missed, or its diagnosis omitted from medical record (13% vs 1%, p=0.016 and 48% vs 15%, p<0.001). In the retrospective study, altogether 149 RSV, 521 influenza A and 155 influenza B hospitalizations were included over four epidemic seasons. The mean attack rates of RSV, influenza A, and influenza B hospitalizations rose with age from 4.1 (range by season 1.9-5.9), 15.4 (12.3-23.3), and 4.7 (0.5-16.2) per 100 000 persons among 18-64 year-olds to 58.3 (19.3-117.6), 204.1 (31.0-345.0) and 60.4 (0.0-231.0) per 100 000 persons among 65+ year-olds and varied considerably between seasons (Figure 1). Attack rates of influenza hospitalizations were high among the 65+ elderly despite more than half of the elderly population being vaccinated against influenza each season. Over four epidemic seasons, the mean attack rates of influenza (A + B) were four to five times higher than those of RSV in all age groups.

**CONCLUSIONS**
We observed a considerable and partially clinically undetected and underreported disease burden of laboratory-confirmed RSV among hospitalized adults. While the attack rates of influenza hospitalizations were higher compared with RSV, RSV and influenza hospitalizations were similar in severity. Missing or underreporting of RSV infections may lead to underestimating its disease burden. Together, RSV and influenza caused a substantial amount of hospitalizations among the elderly, stressing the need for more effective interventions.

![Figure 1. RSV and influenza hospitalizations per 100 000 adults living in the hospital catchment area during epidemic seasons 2016-20](image-url)
Predictors of seeking care for influenza-like illness using a novel digital study design

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Alejandra Benitez (1), Allison Shapiro (2), Hao Xu (3), Victoria Whitehill (1), Ernesto Ramirez (2), Kelly Scherer (2), Luca Foschini (2), Faye Drawnel (4), Vincent Uwachukwu (5), Devika Chawla (1)


**BACKGROUND**

Traditional influenza surveillance systems and real-world data (RWD) studies rely on records from clinical visits for influenza-like illnesses (ILI). This selection bias overlooks individuals with ILI who do not seek healthcare. To address this knowledge gap, we used a person-generated health data (PGHD) approach to study a population of individuals experiencing ILI and identify factors associated with seeking healthcare for ILI.

**METHODS**

We utilized data from two prospective studies, FluStudy and a participatory ILI surveillance program (ISP), to address our research objectives. For both, we identified participants on Evidation's Achievement consumer (mobile app) or studies (web portal) platforms who self-reported ILI during the 2019-2020 influenza season. ILI was defined as having at least one respiratory (e.g. cough or sore throat) and one systemic symptom (e.g. fever or headache). Eligible participants were aged ≥18 years, United States residents, and English speakers. FluStudy participants were additionally required to own and be willing to wear a Fitbit. Survey responses were excluded if implausible or missing. We conducted a log-binomial regression to identify factors associated with seeking healthcare during an ILI.

**RESULTS**

Of the 1,667 FluStudy participants eligible for inclusion in the analysis, the majority were female (92%), aged 18–49 (90%) and white (89%). There were 518 (31%) FluStudy participants who sought care for their ILI. In the log-binomial regression, seeking care was associated with having health insurance (2.14, 95% CI: 1.30, 3.54), having more severe respiratory symptoms (1.53, 95% CI: 1.37, 1.71) and being at high risk of influenza complications due to comorbidities (1.37, 95% CI: 1.20, 1.58).

Of the 47,480 ISP participants included in the analysis, 81% were female, 90% were aged 18–49, and 77% were white. There were 11,426 (24%) participants who sought care. In the log-binomial regression, seeking care was associated with experiencing a higher number of symptoms (RR: 1.23, CI: 1.21, 1.24), being obese (1.20, 95% CI: 1.15, 1.25) and having other risk factors for influenza complications (RR: 1.13, CI: 1.09, 1.17).

**CONCLUSIONS**

Using evidence from two RWD studies, we showed that more than two-thirds of individuals experiencing ILI do not seek healthcare during their illness. This indicates that traditional medical research methods often exclude a majority of individuals experiencing ILI. Care seekers had higher rates of health insurance, higher rates of comorbidities with risks of ILI complications, and more severe ILI symptoms than non-care seekers. In future work, it is crucial to recruit and enroll study cohorts with demographic profiles that are more representative of the underlying population. This PGHD approach helps understand factors associated with seeking care for ILI, which may in turn inform public health policies that increase access to care for individuals with influenza.

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

**Authors:**
- Angel Gil (1), Javier Díez-Domingo (2), Federico Martín-Jarres (3), Esther Redondo (4), Raúl Ortiz de Lejarazu (6), Ionut Pumarela (8), Jaime Carreira de Sousa (7), Carlos Rabaça (8), João Raposo (9), Carlos Robalo Cordeiro (10), Mafalda Carmo (11), Hugo Lopes (12), Geoffrey Bizouard (13), Íñigo María Guillén (14), Catarina Genes (15), Margarida Martins (15), Carlos Cunznan (16), Hélène Briquet (16), Filipe Freies (17)

1: Public Health and Medical Specialties Department, Health Sciences Faculty, Juan Carlos University, Madrid, Spain;
2: Vaccine Research Department, University of Valencia, Valencia, Spain;
3: Pediatría Department, Hospital Clínico Universitario de Santiago, Santiago de Compostela, Spain;
4: International Health Center Madrid Health, City Council of Madrid, Madrid, Spain;
5: Valladolid National Influenza Centre, Hospital Clínico Universitario de Valladolid, Valladolid, Spain;
6: Viralogy Section, Department of Microbiology, Barcelona Centre for International Health Research (CRESIB), Hospital Clinic - Universitat de Barcelona, Barcelona, Spain;
7: Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga Portugal;
8: IQVIA, Lisbon, Portugal;
9: APDP and Nova Medical School, Lisbon, Portugal;
10: Pulmonology Department, Cebreros University Hospital, Portugal;
11: KIVI, Barcelona, Spain;
12: KIVI, Lisbon, Portugal;
13: KIVI, Paris, France;
14: Sanofi Pasteur, Madrid, Spain;
15: Sanofi Pasteur, Lisbon, Portugal;
16: Sanofi Pasteur, Lyon, France;
17: ISCIII, Thaon Department, Centro Hospitalar Universitário de Lisboa Norte, Lisbon, Portugal

**BACKGROUND**
Influenza viruses are responsible for seasonal epidemics causing significant burden to health care systems every winter. Frail people are particularly at-risk of influenza complications, potentially leading to hospitalization or even death, both seldom coded with influenza-specific diagnosis. Statistical modelling techniques - using influenza activity as a key explanatory variable - can be used to estimate annual hospitalizations and deaths associated with influenza. The objective of the study is to estimate the burden of severe influenza, between 2008 and 2018 in Spain and Portugal.

**METHODS**
The excess hospitalization and mortality attributable to influenza in each country was estimated based on time series ecological models and compared for six age groups. The primary predictor of influenza excess hospitalizations and deaths was the weekly incidence rate of influenza-like illness (ILI), which was obtained from the surveillance systems. Poisson cyclic models were used, where age- and cause-specific hospitalization and mortality data were explained by the ILI incidence, time trends and seasonal terms, using a log link. The analysis of excess mortality attributable to influenza used all-cause deaths from the national death certificates, obtained from the National Institutes of Statistics. Excess hospitalization attributable to influenza used cardiovascular or respiratory (C&R) hospitalization data from the National Health Services, coded as ICD-9: 390-459, 460-469; or ICD-10: 100-199, J00-J99. Costs were computed by multiplying the number of estimated excess hospitalizations by the mean cost per hospitalization in each country, by cause and age group. The study comprised ten epidemic seasons (2008/09-2017/18) but excluded the H1N1pd09 pandemic season 2009/10.

**RESULTS**
Mean annual C&R hospitalizations attributable to influenza were estimated at 34,894 in Spain (min: 16,546; max: 52,861) and 5,356 in Portugal (min: 456; max: 8,776). Mean annual excess C&R hospitalization rate per 100,000 was estimated at 75.0 in Spain (95%CI: 63.3-85.3) for all-ages and 335.3 (95%CI: 293.2-377.5) in population aged ≥65 years. In Portugal, the rates were 51.5 (95%CI: 40.9-62.0) and 199.6 (95%CI: 163.9-235.4), respectively. These hospitalizations represented a mean annual cost of 142.9 M€ (min: 68.6 M€; max: 216.6 M€) in Spain and 15.2 M€ in Portugal (min: 1.3 M€; max: 24.9 M€). Population aged ≥65 years accounted for 76.4% of the cost in Spain, and 75.4% in Portugal, followed by population between 50-64 years old (16.2% and 16.0%, respectively). In both countries, the season with the highest estimated all-cause mortality attributable to influenza was 2014/15, with 24,286 deaths in Spain and 5,016 in Portugal, 93.2% and 95.9% of which occurred in people aged ≥65 years, respectively.

**CONCLUSIONS**
Our results improve the understanding of influenza burden, showing the impact of influenza and its complications on hospitals – in terms of public health and economic burden - which is particularly important in the 65 years and over population.

**Funding**
This study was funded by Sanofi Pasteur.
Global patterns of seasonal influenza activity, duration of activity and virus (sub)type and B-lineage circulation from 2010 to 2020

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Patrizio Zanobini (1), Guglielmo Bonaccorsi (1), Chiara Lorini (1), John Paget (2), Mendel Haag (4), Ian McGovern (3), Saverio Caini (5)

1: Department of Health Sciences, University of Florence, Florence, Italy;
2: Netherlands Institute for Health Services Research (Niels), Utrecht, the Netherlands;
3: Seqirus Inc, Cambridge, MA, USA;
4: Seqirus Netherlands B.V, Amsterdam, the Netherlands;
5: Cancer Risk Factors and Lifestyle Epidemiology Unit, Institute for Cancer Research, Prevention and Clinical Network (ISPRO), Florence, Italy

**INTRODUCTION**

Vaccination is the mainstay for preventing influenza infections and complications. Detailed knowledge of influenza strain circulation and seasonality of epidemics is essential to making recommendations about vaccine composition and timing of administration, and therefore to achieving maximal vaccine effectiveness. We performed an updated analysis of the global epidemiology of influenza, with a focus on influenza virus (sub)type circulation patterns and the temporal characteristics (peak timing and duration) of influenza epidemics around the world in the decade following the 2009 H1N1p pandemic up until the emergence of COVID-19 (2010-2020).

**METHODS**

Influenza virological surveillance data were obtained from the publicly available WHO FluNet database on 22nd April, 2021. For each country and season, we determined the proportion of laboratory-confirmed influenza cases caused by the different virus (sub)types and B-lineages. We calculated the month of the primary and secondary epidemic peak by analysing country-specific influenza times-series using the EPIPOI software. We then calculated the duration of the influenza epidemic, defined as the shortest stretch of consecutive weeks that account for at least 75% of all influenza cases reported in the season.

**RESULTS**

The analysis included 4,659,001 influenza cases from 149 countries. Type A and B influenza viruses accounted for 72.5% and 27.5% respectively of all reported influenza cases. The median proportion of influenza A cases was highest in countries in the Southern Hemisphere (81.5%) and lowest in countries of the intertropical belt (73.0%). The median proportion of influenza A cases ranged between a minimum of 60.9% in the 2017 season and a maximum of 88.7% in the 2018 season. The median % of cases accounted for by each influenza virus (sub)type was 29.9% for A(H3N2), 23.4% for A(H1N1), 13.8% for unsubtyped A, 22.8% for uncharacterized B, 5.8% for B Yamagata, and 3.3% for B Victoria.

For Northern Hemisphere countries, the typical timing of the epidemic peak occurred between December and March, while for Southern Hemisphere countries, it took place in July or August. Much more variability emerged in the intertropical belt where some countries were characterized by small-amplitude primary peaks and well-defined secondary peaks. The median duration of influenza epidemics by latitude is shown in figure 1.

**CONCLUSIONS**

We described the global variability in timing, duration, and strain circulation of influenza epidemics during 2010-2020. This work provides an important reference to compare influenza seasonality before and after the COVID-19 pandemic, and will therefore have important implications for vaccination programmes in the coming years.

![Figure 1: Median duration of the influenza epidemics by country latitude.](image-url)
A statistical model for estimating age-specific influenza-associated excess mortality in Germany from 1996 to 2018

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Christian Schindler (1), Nils Kossack (1), Oliver Damm (2), Rolf Kramer (2), Tonio Schoenfelder (1,3)

1: WIG2 GmbH, Germany; 2: Sanofi Aventis Deutschland GmbH, Berlin, Germany; 3: Chair Health Sciences/Public Health, Faculty of Medicine, Technische Universität Dresden, Dresden, Germany

**BACKGROUND**

Mortality due to influenza is often underestimated, since in contrast to many other diseases, influenza is not always reported as the cause of death, and a significant proportion of attributable mortality arises from influenza’s contribution to other conditions (e.g., cardiovascular diseases). It has therefore become common practice to estimate influenza-associated excess mortality by statistical methods, using all-cause deaths or other mortality definitions (e.g., cardiorespiratory deaths). The Robert Koch Institute (RKI) uses a method based on the annual distribution of monthly relative all-cause mortality (relative mortality distribution method, RMDM). However, no age-specific estimates have been published for Germany so far, and currently available estimates are based on monthly data only. These disadvantages are addressed in our study.

**METHODS**

The study was designed as a retrospective data analysis based on secondary data of the entire population in Germany from 1996 to 2018. We adopted the RKI’s approach of estimating excess mortality with the RMDM but used weekly and age-specific death data to calculate the number of excess deaths for four age groups. Influenza activity was determined from official surveillance reports, while weekly and age-specific all-cause death data were obtained from the Research Data Centers of the Statistical Offices of the Federation and the Federal States (FDZ). The expected mortality (without influenza in the background) was calculated by multiplying the weekly proportions by the total annual mortality. Excess mortality was calculated by subtracting observed mortality in influenza seasons from this expected mortality. A conservative excess estimate was obtained by subtracting one standard deviation of the differences between observed and expected mortality during influenza-free calendar weeks.

**RESULTS**

Total influenza-associated excess deaths (i.e., covering all age groups) were comparable to RKI's estimates for most seasons. The majority of excess deaths occurred in patients aged 60+ years (up to 98% in the seasons shown in Table 1).

<table>
<thead>
<tr>
<th>Season</th>
<th>RKI (weekly deaths)</th>
<th>Total</th>
<th>0-34 years</th>
<th>15-24 years</th>
<th>25-64 years</th>
<th>65+ years</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016-17</td>
<td>2,140</td>
<td>2,337</td>
<td>3</td>
<td>11</td>
<td>62</td>
<td>2,042</td>
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<tr>
<td>2017-18</td>
<td>20,790</td>
<td>20,951</td>
<td>43</td>
<td>88</td>
<td>805</td>
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<tr>
<td>2018-19</td>
<td>6,179</td>
<td>6,179</td>
<td>5</td>
<td>16</td>
<td>0</td>
<td>5,934</td>
</tr>
<tr>
<td>2019-20</td>
<td>21,900</td>
<td>15,874</td>
<td>19</td>
<td>22</td>
<td>930</td>
<td>14,703</td>
</tr>
<tr>
<td>2020-21</td>
<td>6,583</td>
<td>6,583</td>
<td>24</td>
<td>73</td>
<td>132</td>
<td>5,367</td>
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<tr>
<td>2021-22</td>
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<td>25,766</td>
<td>34</td>
<td>57</td>
<td>496</td>
<td>23,309</td>
</tr>
<tr>
<td>2022-23</td>
<td>25,630</td>
<td>25,630</td>
<td>28</td>
<td>67</td>
<td>1,338</td>
<td>24,113</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

This is the first study that provides age-specific estimates of influenza-associated excess mortality for Germany. Results clearly show that the main burden of influenza-associated mortality is in the elderly to whom prevention and control measures should be prioritized.

**Funding** The study was funded by Sanofi-Aventis Deutschland GmbH.
Costs-effectiveness of influenza vaccination with a high dose quadrivalent vaccine of the Belgian elderly population

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

Caroline de Courville (1), Pierre Chevalier (2), Matthias Borms (2), Hélène Bricout (1), Christine Petit (3), Fabián Alvarez (1)

1: Sanofi Pasteur, 69007 Lyon, France;
2: IQVIA, 1930 Zaventem, Belgium;
3: Sanofi Pasteur, 1831 Diegem, Belgium

**BACKGROUND**
We assessed the impact of introducing Efluelda® to Belgium, a high dose quadrivalent inactivated influenza vaccine (HDQIV), compared to the standard dose influenza vaccines (SDQIV), the current standard of care, for the protection against influenza and associated burden in individuals aged 65 years and older. The analysis estimates savings for both healthcare resources consumption and costs, as well as cost-effectiveness associated with the switch in the population aged 65 years and older.

**METHODS**
A decision tree model, with a one-year time horizon, was used to compare both, health outcomes and costs engendered by influenza in adults aged 65 years or older vaccinated with HDQIV vs. SDQIV. The decision tree allows for estimation of – for each vaccine – the influenza cases, general practitioner (GP) visits, emergency room (ER) visits, hospitalizations, and influenza-related deaths.

The number of cases for each vaccination strategy is calculated from the attack rate of an unvaccinated cohort, the coverage rate, and the efficacy of each vaccine. In the base case an influenza attack rate of 7.2% for unvaccinated, and a vaccine coverage rate of 53.1% were assumed. Influenza-related hospitalizations were assessed in two different scenarios: admissions due to laboratory-confirmed influenza only (conservative), and hospitalizations due to respiratory events possibly attributable to influenza (base case). The second scenario was used as base case to avoid underestimation of the influenza-related hospitalizations. As measured in the pivotal randomized clinical trial, relative efficacy of HDQIV vs. SDQIV was assumed to be 24.2% against lab-confirmed influenza, and 25.3% against broad influenza hospitalizations.

The probabilities of emergency room (ER) and general practitioners (GP) visits amongst the influenza cases, as well as all demographic, epidemiological, population utilities and economic inputs were based on Belgian data. The economic evaluation used healthcare payer perspective.

**RESULTS**
In the base case, the switch from SD-QIV to HD-QIV in adults over 65 years would prevent 10,103 influenza cases, resulting in averted 4,254 GP and 81 ER visits, 1,849 hospitalizations and 260 deaths each year. Compared to the strategy with SD-QIV vaccine, Efluelda® would be cost-effective up to a price of 90 € at a willingness to pay (WTP) threshold of € 35,000/QALY. Deterministic and probabilistic sensitivity analysis were performed, for which Efluelda® has a 100% probability of being cost-effective at the WTP threshold.

![Cost-effectiveness Plane](image)

Figure: Cost-effectiveness plane for the incremental difference of HDQIV vs. SD-QIV

Additional scenarios were tested with analogous results. In the most conservative scenario where only the hospitalizations due to lab-confirmed influenza were considered the cost-effective price for Efluelda would be 84€ at the WTP threshold.

**Conclusion**
A switch to HD-QIV from SD-QIV in the elderly Belgian population would contribute to reach public health objectives, reducing influenza-related mortality and healthcare resources consumption, while being a highly cost-effective strategy.
The Spectrum of Influenza in Children in Managua, Nicaragua

**Topic:** Epidemiology, surveillance and modelling of influenza, RSV disease and COVID-19, including virus evolution and strain selection

**Authors:** Gregory Hoy (1), Guillermina Kuan (2,3), Roger Lopez (2), Nery Sanchez (2), Brenda Lopez (2), Sergio Ojeda (2,3), Hannah Maier (1), Steph Wraith (1), Lora Campredon (1), Angel Balmaseda (2,4), Aubree Gordon (1)

1. Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Michigan, USA;
2. Sustainable Sciences Institute, Managua, Nicaragua;
3. Centro de Salud Sócrates Flores Vivas, Ministry of Health, Managua, Nicaragua;
4. Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministry of Health, Managua, Nicaragua

**BACKGROUND**

Though influenza is common among children globally, understanding of the breadth of clinical presentation of influenza remains elusive, and the frequency of asymptomatic and mildly symptomatic illness is poorly understood. Furthermore, there is little consensus on the importance of influenza type, subtype, and lineage on clinical presentation and prognosis. This study seeks to establish the full spectrum of influenza illness in children and explore associations between clinical presentation and influenza virus type, subtype, and lineage.

**METHODS**

The Nicaraguan Pediatric Influenza Cohort Study (NPICS) is a prospective cohort study based in Managua, Nicaragua. Children present to the study health center when ill and are tested for influenza using real time reverse-transcription polymerase chain reaction. Children aged 0 to 14 years with one or more PCR-confirmed influenza episode between 2011 and 2020 are included in the study. Data on clinical presentation and sequelae were obtained from clinical visits occurring up to ten days before and thirty days after the positive PCR. Age-adjusted associations between virus type/subtype/lineage and aspects of the clinical presentation were calculated using logistic regression. A subset of children under active surveillance for influenza are also being analyzed to allow for a characterization of the full spectrum of infection.

**RESULTS**

A total of 2,140 influenza infections across 1,394 children were identified, with an average age at infection of 5.87 years. Fever, cough, and rhinorrhea each occurred in over 80% of cases. No other symptom occurred in over 50% of cases. Acute otitis media (AOM) and pneumonia were the most common sequelae observed, occurring in 3.3% and 2.4% of cases, respectively. Sinusitis and febrile seizures occurred in less than 1% of cases.

Clinical presentation was largely consistent between type, subtype, and lineage. Cough and rhinorrhea were significantly more likely to occur from Influenza A vs. Influenza B, and cough was less likely to occur from influenza A H3N2 than influenza A H1N1pdm. Both hospitalization (AOR = 0.271, 95% CI 0.117-0.629) and AOM (AOR = 0.501, 95% CI 0.288-0.872) were significantly less likely to occur with H3N2 than H1N1pdm, largely driven by children under the age of 2.

**CONCLUSIONS**

The clinical presentation of medically attended influenza varied greatly and relatively few symptoms consistently occurred, a result that may help explain the difficulty of diagnosing influenza clinically. Influenza sequelae were uncommon, and this study better captures the true burden of post-influenza sequelae when compared to hospital-based studies. The clinical presentation of influenza in children is largely consistent across type and subtype, though H1N1pdm appears to confer greater risk of hospitalization and AOM, especially in children under the age of two. Further analyses are underway to better describe the frequency of asymptomatic and sub-clinical influenza illness in children.
Estimating the impact of influenza infections on health status using the Clinical Risk Groups morbidity-based stratification system

**Topic:** Human influenza, RSV disease and COVID-19 challenge studies

Cintia Muñoz-Quiles (1), Mónica López-Lacort (1), Carlos Vergara-Hernández (1), Javier Díez-Domingo (1,2), Alejandro Orrico-Sánchez (1,2)

1: Vaccine Research Department, FISABIO-Public Health, Valencia, Spain.;
2: Universidad Católica de Valencia San Vicente Mártir, Spain.

**BACKGROUND**
The Clinical Risk Groups (CRGs) constitute a population stratification system based on morbidity which classifies patients into mutually exclusive categories. It was implemented in the Valencia Region Health System in 2010. CRGs capture the resource utilization of all inpatient and ambulatory encounters and assign each person to a health status from healthy to catastrophic, with a severity level for each chronic condition registered. The CRG assignment for an individual is updated monthly. Our objective is to evaluate the CRGs as a tool to measure the impact of influenza on the health status of patients older than 50 years.

**METHODS**
A population-based study was performed using real-world data from the Valencia Health System Integrated Database (VID), in Spain. All subjects over 50 years of age registered in the public health system from January 1st 2013 to December 31st 2018 were included. Influenza cases were defined as all ambulatory and specialist visits with the influenza ICD-9 and ICD-10 codes. Two study designs were implemented: 1) Case-control analyses, where the number of changes in CRGs to a worse health status (higher CRG) during the six-month period "post-influenza" were compared between cases and controls (1:3 ratio, matched by sex, age, health area, month and base CRG); 2) Self-control analyses, where the number of changes in the CRGs to a worse health status were compared between the six months pre- and the six months post-influenza periods in the cases. Relative risks (RR) for both approaches were calculated using a negative binomial regression model with a random effect on the subject (self-control analysis) or the case-control matched group (case-control analysis, which was adjusted by age, sex, cardiovascular disease, chronic obstructive pulmonary disease, diabetes mellitus, obesity, chronic kidney disease, chronic liver disease, immune disorders and number of comorbidities).

**RESULTS**
The study population consisted of 3,438,974 subjects of which 101,332 had an influenza diagnosis code during the study period. After matching, 99,155 cases and 329,091 controls were included. In the case-control analysis, the RR of having changes in CRGs to a worse health status was 18% (95% CI: 1.16–1.20) higher in cases than in controls during the six-month period post-influenza diagnosis. In the self-control analysis, the RR of having changes in CRGs to a worse health status in cases was 27% (95% CI: 1.24; 1.30) higher during the six-month period post-influenza than during the six-month period pre-influenza diagnosis.

**CONCLUSIONS**
The diagnosis of influenza seems to be related to a worsening of the health status that is detectable by measuring the number of changes in CRGs. CRG classification system represents a tool to measure the impact of preventable infectious diseases as influenza on the population morbidity.

**Declarations**
Funding: This study was funded by Sanofi Pasteur within a Chair agreement between FISABIO, Universidad Católica de Valencia San Vicente Mártir and Sanofi Pasteur Spain.
Characterization of antibody response in asymptomatic and symptomatic SARS-CoV-2 infection

**Topic:** Innate and adaptive immunity towards influenza, RSV disease and COVID-19

Serena Marchi  
University of Siena, Italy

**BACKGROUND**

Mechanism of SARS-CoV-2 infection, protection or rapid evolution until fatal outcome of the disease remains poorly understood. Moreover, the recent spread of new variants, carrying several mutations in the spike protein, could impact on immune protection elicited by natural infection.

To elucidate the dynamics of humoral response in SARS-CoV-2 asymptomatic and symptomatic infections, we investigated the time course of antibody response in hospitalized COVID-19 patients and asymptomatic subjects. In addition, the neutralizing activity against the B.1.1.7, B.1.1.28.1, and B.1.351 variants was evaluated.

**METHODS**

Serum samples were collected from 42 hospitalized COVID-19 patients at 6 different time points (hospital admission, day 2, day 6, day 12-14, day 18-20, day 27-30) and from 25 asymptomatic subjects at 3 different time points (at the time of positive swab, 2 months, 6 months). Samples were tested by commercial ELISA for SARS-CoV-2 spike protein IgA, IgM, and IgG and nucleoprotein IgG, and by micro-neutralization assay. Samples for each patient were selected at three time points (hospital admission, highest neutralizing antibody titre against Wuhan strain, and the last sample available during hospital stay) and further tested against B.1.1.28.1 and B.1.351 viruses by micro-neutralization assay.

**RESULTS**

In patients, titres increased for all antibody classes including neutralizing antibody from day 6 to day 18-20 but at day 27-30 started to decline. A high correlation between spike and nucleoprotein antigens and among antibody classes was found. No significant difference in antibody titres at baseline and by peak antibody level was found between recovered and deceased.

64.0% of asymptomatic subjects were negative to any antibody at any time point. Asymptomatic subjects with positive antibody level had titres well below patients, and neutralizing antibodies were found only in 2 subjects.

A significant decrease of neutralizing antibody titre for all three variants was observed at any time point. At discharge/deceased, 59.5% of patients showed a decrease against the B.1.1.7 variant, 83.3% against the B.1.1.28.1 variant, and 90.5% against the B.1.351 variant with respect to the Wuhan strain.

**CONCLUSIONS**

Our results highlight that COVID-19 patients produce an antibody response to SARS-CoV-2 regardless the outcome. The peak is reached by 3 weeks from hospital admission followed by a sharp decrease. On the contrary, only few asymptomatic subjects develop antibodies at detectable levels, though lower compared to COVID-19 patients, raising the question about the protection of these subjects against reinfection. Moreover, the reduction of neutralizing antibody titres against all tested variants, and in particular B.1.1.28.1 and B.1.351 ones, suggests that previous symptomatic infection might be not fully protective to exposure of SARS-CoV-2 variants carrying a set of relevant spike mutations.
Lessons learned from serological studies of influenza A H1N1 pandemic and seasonal vaccination of healthcare workers in Bergen, Norway

**Topic:** Innate and adaptive immunity towards influenza, RSV disease and COVID-19

Håkon Amdam, Anders Madsen, Fan Zhou, Amit Bansal, Mai-Chi Trieu, Rebecca Cox

University of Bergen, Norway

**BACKGROUND**
The ongoing COVID-19 pandemic has shown the importance of vaccination of front-line health care workers (HCW) to prevent infection and protect their patients. During the last pandemic in 2009, the novel influenza A H1N1 virus emerged and continued to circulate being included in the seasonal influenza vaccine up to the 2016/2017. HCW were vaccinated with the AS03-adjuvanted pandemic influenza H1N1 vaccine, and were followed in a longitudinal five year clinical study. HCW were offered seasonal influenza vaccine each season, but vaccination was voluntary not mandatory. The aim of this study was to evaluate the influenza specific antibody response after pandemic and seasonal influenza vaccinations in HCW with different vaccination histories.

**METHODS**
Fifty-five HCW at Haukeland University Hospital, Bergen, Norway were immunized with the AS03-adjuvanted H1N1pdm09 vaccine in 2009 and divided into groups according to their vaccination history; the single group (1 pandemic vaccination), the occasional group (2-4 vaccinations) and the repeated group (5 repeated vaccinations). Serum samples were collected at regular intervals pre- and post (at 21 days, 3, 6, and 12 months) vaccination, or annually before the influenza season from 2010 in the single group. Haemagglutinin specific responses were investigated using the haemagglutination inhibition (HI), ELISA, and microneutralisation (MN) assays.

**RESULTS**
Pandemic vaccination induced an increase in the HA-specific antibodies in all HCW measured by all assays. The adjuvanted pandemic vaccine induced a durable antibody response in the single group, with protective HI antibodies (HI titre ≥40) detected in 6 of 10 HCW measured at 5 years. The occasional group generally had higher antibody responses compared to the repeated group. HCW who were not vaccinated in 2012 had significant increases in their antibody titres at 21 days post-2013 IIV vaccination, compared to HCW vaccinated in both 2012 and 2013 suggesting that the interval between vaccines influences the antibody response to the same strain.

**CONCLUSIONS**
We observed higher antibody responses in the occasionally vaccinated HCW compared to the repeatedly vaccinated HCW post-2013 IIV vaccination, but the benefit to the two other vaccine strains A/H3N2 and B was not measured. Our findings contribute to our understanding of antibody responses to the same strain after priming with an adjuvanted pandemic vaccine followed by repeated annual vaccination. More work is needed to understand the broader immunological impact of repeated annual vaccination to provide optimal protection for HCW.
Study of neuraminidase inhibiting antibodies to assess immunity to influenza after infection and vaccination

**Topic:** Innate and adaptive immunity towards influenza, RSV disease and COVID-19

Yulia Desheva (1), Nadezhda Petkova (1), Igor Losev (1), Andrey Rekstin (1), Chih Hsuan Tsai (2), Yu-Chan Chao (2)

2. Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan

**BACKGROUND**
NA-based immunity may be important for protection against novel antigenic influenza variants. Therefore, the detection of neuraminidase inhibiting (NI) antibodies may improve the study of the immunogenicity of newly developed influenza vaccines. The lack of experimental and clinical data in this area makes it difficult to quantify the protective efficacy of NI antibodies and, therefore, to develop criteria for assessing the immunogenicity of an influenza vaccine based on the assessment of NI antibodies. The aim of the study was to evaluate antibodies to influenza NA and to assess different methods for detecting NI antibodies.

**METHODS**
The study studied the blood serum of the patients of the Medical Scientific Center (IEM) collected in 2018-2019 and the sera of patients who had influenza type A collected in 2017 at the Clinical Infectious Diseases Hospital. The study was approved by the Local Ethics Committee at the FSBSI "IEM" (04/23/2019). We used different methods to detect antibodies to NA in the persons vaccinated against influenza or those who had an influenza infection. The levels of NI antibodies were assessed in the Enzyme-Linked Lectin Assay (ELLA) using reassortant H3N1 or H3N2 viruses. Also, we used Enzyme-Linked Immunosorbent Assay (ELISA) based on Spodoptera frugiperda cell line Sf21 expressing the full-length NA of the influenza A/California/07/2009 (H1N1)pdm09 virus fixed to the bottom of 96-well ELISA panels.

**RESULTS**
Using ELLA, it was shown that the lowest levels of antibodies to NA subtype N1 were noted in the group of volunteers born during the circulation of influenza viruses of subtypes A/H2N2 and A/H3N2, and the lowest levels of antibodies to NA subtype N2 were noted among people born from 1977 to 2000. During immunization with trivalent live influenza vaccine (LAIV), the determination of NI antibodies made it possible to identify additional seroconversions to A/H1N1 and A/H3N2 viruses. The moderate levels of correlation between NI and neutralizing antibodies indicates that part of NI antibodies have virus-neutralizing properties.

A protocol has been developed for an ELISA with blood sera of people vaccinated against influenza or having had influenza infection, based on the NA of the influenza virus expressed on Sf21 cells. It has been shown that NA expressed on baculovirus exhibits enzymatic activity no lower than that of whole virus NA. When analyzing the antibody increases in paired sera of patients with confirmed influenza infection, a high correlation was found between the data obtained using ELLA and ELISA (r = 0.79). ELISA test proved to be specific and quite convenient, since the transformed culture sorbed on plastic panels is stable during transportation.

**CONCLUSIONS**
Our research has shown good results for three evaluated tests (ELLA, ELISA and microneut). These tests can be used to determine the antibody levels needed to protect against novel antigenic variants of influenza viruses.

This research was supported by Russian Foundation for Basic Research, grant number 20-54-SS2006 MHT_a_7.
**Differences in the humoral response after vaccination against COVID-19 in nursing-home residents**

**Topic:** Innate and adaptive immunity towards influenza, RSV disease and COVID-19

Iván Sanz-Muñoz (1), Rosa López-Mongil (3), Diana Pérez-San José (1,3), Laura Sanchez-de Prada (1,3), Javier Castrodeza-Sanz (1,3), Sonia Tamames-Gómez (2), José María Eiros-Bouza (1,3), Raúl Ortiz de Lejarazu-Leonardo (1)

1: NIC Valladolid-HCUV, Spain; 2: Epidemiology Department, Ministry of Health of Castile and Leon, Spain; 3: University of Valladolid, Spain

**BACKGROUND**

The aim of this study is to describe the serological profile of the response to COVID-19 vaccines and its relationship to various clinical and demographic factors in nursing-home residents.

**METHODS**

A prospective observational study was carried out in which 98 individuals older than 65 years institutionalized in two nursing homes, vaccinated with BNT162b2 (Comirnaty) were included. IgG antibodies against three SARS-CoV-2 virus antigens were analyzed: S, RBD (Receptor Binding Domain) and N. The first serum was collected 20 days after the first dose, and the second one, one month after the second dose. A modified ELISA (Luminex) was used for detecting and quantifying antibodies. Serological, clinical and demographic parameters were analyzed; previous positive result for SARS-CoV-2 (PCR or Rapid Antibody Test: RAT), age, sex, obesity, vitamin D, chronic kidney failure, diabetes, dementia, sarcopenia, frailty and malnutrition.

**RESULTS**

Mean Age was 83.3 years. Men was 41.8% of people included (n=41). No significant differences were found in the proportion of positive and negative cases for SARS-CoV-2 between both sexes (Chi-square; p>0.05). After the first dose, 55.1% of individuals showed a low response to the vaccine (Low Responders-LR) and 44.9% showed a high antibody response (High Responders-HA). After the second dose, the antibodies were between 3 and 7 times higher in the HR than in the LR. The binary logistic regression showed that the main factor associated with the High Response was having a previous positive test for SARS-CoV-2 (PCR neither RAT; \( \chi^2 \), p<0.05). When these two factors were eliminated from the analysis, dementia (OR=5.1, CI95%;1.60-16.5), frailty (OR=3.3, CI95%;1.11-10.3) and malnutrition (OR=11.3, CI95%;1.1-119.9) were significantly more frequent in the LR (logBin; R2=0.390, p<0.05).

**CONCLUSIONS**

The serological response to the BNT162b2 vaccine in nursing-home residents depends mainly on previous exposure to SARS-CoV-2, being higher in previously infected individuals. Factors such as dementia, frailty and malnutrition are associated with a worse response to the vaccine. Revaccination with a third dose in elderly people who have not previously suffered COVID-19 is necessary to complete protection.
Durability of antibody immune responses to SARS-CoV-2 after natural infection and vaccination

**Topic:** Innate and adaptive immunity towards influenza, RSV disease and COVID-19

Irina Isakova-Sivak, Victoria Matyushenko, Ekaterina Stepanova, Arina Goshina, Anna Chistiakova, Polina Prokopenko, Ivan Sychev, Svetlana Donina, Daria Mezhenskaya, Larisa Rudenko

**Institute of Experimental Medicine, 197376 Saint Petersburg, Russian Federation**

**BACKGROUND**
Assessment of the levels of antiviral immunity in patients who recovered from the new coronavirus infection, as well as in those vaccinated with COVID-19 vaccines, is necessary to monitor the immunological status of the population and the effectiveness of vaccines used in practice. In this study, we assessed the durability of RBD-specific serum IgG and neutralizing antibody responses after COVID-19 and vaccination with the Sputnik V vaccine.

**METHODS**
Sixty COVID-19 convalescents and forty subjects immunized with Sputnik V donated whole blood at different periods post-infection or vaccination. All participants signed an informed consent before sample collection. The COVID-19 recovered patients were divided into two groups based on the severity of the disease symptoms, with the disease onset ranging from May 202 to February 2021. The study population also included 20 naive subjects. The levels of IgG antibody to recombinant RBD protein and neutralizing antibody were measured in serum samples using standard ELISA and MN [Amanat et al., Curr. Protoc. Microbiol. 2020] assays, respectively. Both assays involved Wuhan variant of SARS-CoV-2.

**RESULTS**
Virus-specific IgG and neutralizing antibody levels were maintained for over 12 months in moderate-to-severe COVID-19 cases (Figure, upper panel). Serum IgG antibody were also long-lived in mild COVID-19 cases, with MN50 titers remaining at significant levels over 6 months after recovery (Figure, middle panel). Sputnik V induced high levels of RBD-specific IgG antibody that persisted for several months after vaccination in all individuals. Neutralizing antibody levels were detected in 31 of 40 vaccinated subjects, and these antibodies rapidly declined after vaccination, suggesting that the vaccine-induced protective immunity was not long-lasting.

**CONCLUSIONS**
Natural SARS-CoV-2 infection induces long-lasting antibody immune responses, with clear evidence that neutralizing antibodies persist longer in patients recovered from severe disease, compared to the mild infection. Sputnik V induced long-lived RBD-binding IgG antibodies, although neutralizing antibody levels did not persist, suggesting that a booster vaccine dose might be required to afford clinical protection against natural infection.

**Funding** The study was funded by the RSCF grant №21-75-30003.
Lambda Interferons are specific suppressors of RNA-viruses

**Topic:** Innate and adaptive immunity towards influenza, RSV disease and COVID-19

**Alexey Lozhkov (1), Ekaterina Romanovskaya Romanko (2), Marina Patrakhova (2), Irina Baranovskaya (1,2), Maria Sergeeva (1,2), Sergey Kiotchenko (2), Andrey Vasin (1,2,3)**

1: Peter the Great St. Petersburg Polytechnic University, Russian Federation;
2: Smorodintsev Research Institute of Influenza, Russian Ministry of Health, 197376 St. Petersburg, Russia;
3: Scientific and Educational Center for Biophysical Research in The Field of Pharmaceuticals, Saint Petersburg State Chemical Pharmaceutical University, 197022 St. Petersburg, Russia

**BACKGROUND**

Mucous membranes of the respiratory tracts are the primary tissue targets of type III interferons (IFN-λ) that are crucial for the innate immune response. Although IFN-λ are considered to be universal antivirals, data on their activity against DNA-viruses is limited.

**METHODS**

The A549 (CCL-185) and Vero (CCL-81) cell line was obtained from ATCC. Balb/c mice were used for in vivo experiments.

Viral strains were obtained from the Virus and Cell Culture Collection of the Smorodintsev Research Institute of Influenza. SARS-CoV-2 virus (hCoV-19/Russia/SPE-RII-524V/2020) was isolated from a patient oropharyngeal swab in Vero cells. Experiments with SARS-CoV-2 were carried out in A.A. Smorodintsev Department of Virology of the Institute of Experimental Medicine. Virus titer was determined on the third day after infection.

**RESULTS**

The antiviral effect of IFN-λ1 against influenza A virus (IAV) was only observed with the ‘preventive’ and ‘preventive/therapeutic’ administration regimens. In the ‘preventive/therapeutic’ scheme, viral genome expression was significantly reduced.

Administration of murine IFN-λ3 according to a ‘preventive’ scheme in vivo can reduce mortality and viral load on the third day after lethal IAV infection. However, with human IFN-λ1, these protective effects were not obvious (figure 1A).

Antiviral activity against SARS-CoV-2 was evaluated in Vero cells. A significant decrease in TCID50 was observed in ‘preventive’ (3.5 logarithm) and ‘preventive/therapeutic’ (3.5 logarithm) regimens. At concentration of 500 ng/ml, statistically significant decrease in the SARS-CoV-2 titer was noted in the ‘therapeutic’ regimen (figure 1B).

IFN-λ1 did not show antiviral activity against type 5 adenovirus (AdV).

We assessed the dynamics of IFNL and ISGs production in response to infection of A549 cells with respiratory viruses (IAV(H1N1)pdm09, IAV(H3N2), IBV, RSV AdV, and AdV). While infection with RNA viruses led to a significant IFNs expression, AdV elicited only a weak increase in IFNL and IFNB mRNA (figure 1C). The level of ISGs mRNA (IFIT1, MxA, SOCS-1), as well as cytosolic RNA-sensors (RIG-I, MDAS), significantly increased only in case of RNA viruses.

**CONCLUSIONS**

IFN-λ1 antiviral activity against IAV and SARS-CoV-2 was shown. Despite the hypothesis that IFN-λ is a universal defense mechanism of the innate immune system, there was no antiviral effect against AdV. Infection of A549 cells with AdV does not activate intracellular IFN-dependent defense mechanisms.

![Figure 1](image_url)

**Figure 1.** IFN-λ elicited antiviral activity against IAV in vivo (A) and SARS-CoV-2 (B), while infection with AdV did not induce IFN and ISGs expression (C). Research was supported by Russian State Assignment for fundamental research (0784-2020-0023).

**Topic:** Pandemic Preparedness Planning in Peacetime

**Faisal Irshad**, Aurooj Un Nisa, Rashmi Sharma, Sumit Gandhi

1: CSIR-Indian Institute Of Integrative Medicine, India; 2: Watson-Crick Centre for Molecular Medicine, Islamic University of Science and Technology (IUST), Awantipora, J&K, India

**BACKGROUND**

Several vaccines against Influenza illness and COVID-19 have been licensed and are being delivered internationally in various locations. The general public's attitudes, and impressions of influenza vaccines, on the other hand, are poorly understood which is found to be one of the causes that some people are hesitating in taking of COVID-19 Vaccine. The study's goal was to learn more about the community's knowledge, attitudes, and views about influenza vaccines and how it affects COVID-19 vaccination uptake in Jammu and Kashmir.

**METHOD**

An anonymous population-based e-survey was performed among general persons as a preliminary investigation. In this study, 1125 people participated voluntary, among (64.6% male; mean age: 27.19 ± 5.0 years; age range: 18-68 years). A semi-structured, self-reported questionnaire with informed consent. There were 23 questions divided into 4 parts. The first section dealt with issues of personal demographics. The remaining 3 sections included the knowledge of the influenza and COVID-19 disease, vaccination, attitudes and behaviours.

**RESULT**

Our findings suggest, as soon as the National Vaccination Strategy was established, 51% of our sampling group were willing to take COVID-19 vaccine, and 59.3% advised their families and friends that they should receive this vaccine. In addition to this, the study also shows that there is more or less effect of Influenza vaccine among some people who are not willing to take the vaccine because of the ineffectiveness of Flu Vaccine and some faced severe side effects after being vaccinated against Influenza, this is somehow threat among people who are not willing to get vaccinated for COVID-19. Pregnant women were less willing to receive the vaccine because of unawareness of safety and effectiveness. Our study showed that males were more willing to be vaccinated compared with females. This is believed to be because women are more concerned about adverse side effects of the vaccine because of maternity issues at present or in future. Survey about vaccination conducted among the Kashmir region, females were found to be more knowledgeable and have positive practices and attitudes toward non-pharmaceutical preventive interventions. In one case, the people advice to enhance the vaccination absorption were flu shots for life-long protection, increasing awareness, free cost availability and effective vaccine. Our study shows that less number of people are aware about influenza vaccination than COVID-19 vaccination, which is aligned with comparable studies, showing information has considerable impacts and has a direct effect on attitude on preventive measures through believing.

**CONCLUSION**

The Influenza illness and COVID-19 pandemic continues to raise worldwide catastrophes for life and livelihoods and a possible hope for the future with Influenza and COVID-19 vaccination. This study has shown insufficient understanding but more favourable views about Influenza and COVID-19 in Jammu and Kashmir. The results indicate quick initiatives in health education and more exact information by the health authority should be provided and publicised. In order to decrease the hesitation of the vaccine which has been promoted and enabled with disinformation in the media, policymakers should take efforts in ensuring appropriate understanding, positive attitudes, and views of COVID-19. The government should implement creative risk communication techniques in order to meet all the resistive strategies of the public highlighting in this study, with a major and moderate rate of Influenza and COVID-19 vaccination rejection by participants respectively, driven largely by friends and social media.
Pandemic Preparedness – Have We Got It Right?

**Topic:** Pandemic Preparedness Planning in Peacetime

**William Cracknell** (1), Ray Longstaff (2), Dirk Hofmann (3), Allen Bolands (4), Ranbir Bahra (5)

1. Independent consultant, Australia; 2. Seqirus UK Ltd., United Kingdom; 3. Seqirus GmbH, Germany; 4. Seqirus Pty Ltd., Australia; 5. Independent consultant, United Kingdom

The world is gripped by pandemic. In 2019, before the outbreak of COVID-19, the Global Health Security Index report found, in examining the pandemic preparedness of 195 countries, that, 'National health security is fundamentally weak around the world. No country was fully prepared for epidemics or pandemics, and every country had important gaps to address.'

Whilst elements of the global response to COVID-19 have been impressive – particularly vaccine development - global preparedness for the next pandemic remains sub-optimal. What can be done to improve it? We must change the way we look at the challenge of a pandemic. A pandemic is a paradox – inevitable but unexpected! It engenders fear and uncertainty, with profound physical, psychological, social and economic effects. To be truly prepared we need strategies to both predict and get in front of the disease quickly.

Comprehensive surveillance intelligence is essential to recognise emerging threats. Whilst SARS-CoV-2 and other coronaviruses are of significant concern, the most significant is still influenza. WHO has maintained its effective GISRS capability for over 50 years. Other complementary initiatives such as the Rockefeller Foundation’s Pandemic Prevention Institute and the WHO Hub for Pandemic and Epidemic Intelligence aim to monitor and identify global threats within 100 days of first emergence.

Critically, being fully pandemic prepared requires rapid population-wide access to an effective vaccine. The speed at which a pandemic vaccine can be delivered depends on both external factors and manufacturer dependant-factors. Well-proven technologies already exist and familiarity with seasonal flu vaccinations may facilitate uptake. Importantly, manufacturers have developed sophisticated supply chains and preparedness procedures to switch operations rapidly from seasonal to pandemic flu vaccine. Regulators in some jurisdictions, have established expedited pathways to swap pandemic strains into already approved vaccines. Manufacturing capacity is a high demand, highly limited resource in a pandemic. Governments should plan for access to available capacity as a core part of their pandemic strategy.

Whilst potentially pathogen agnostic technology such as mRNA will prove useful with flu and non-flu pathogens, they still require time-consuming trials to show safety and efficacy and will possibly be subject to the same current hesitancy issues. In the long-term it will be important to have diverse platforms to mitigate risk and ensure rapid availability of supplies for the global population. Until new technologies are proven, our fastest response will remain in currently licenced and proven influenza platforms where first batches could be ready for release within 100 days of a pandemic declaration.

Established structures and framework for influenza pandemic preparedness remain a highly relevant blueprint. New policies based on the COVID-19 experience should build upon and embed those structures to demonstrate true preparedness when the next pandemic occurs.

[4] Toward Preventing Pandemics in the First 100 Days – The Rockefeller Foundation
EPIDEMIOLOGICAL SITUATION OF AVIAN INFLUENZA IN UKRAINE DURING 2020-2021 (1st quarter)

Topic: Pandemic threats from the animal world

Maryna Sapachova, Mykola Sushko, Svitlana Mandyhra, Volodymyr Zahrebelnyi, Olha Chechet

State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, Ukraine

BACKGROUND

Avian influenza viruses (AIVs) are spread globally by wild migratory birds that are reservoirs of AIVs. Epidemics of highly pathogenic avian influenza (HPAI) can devastate the poultry industry and result in severe trade restrictions. Many routes of wild migratory birds pass through the territory of Ukraine, therefore studying the circulation of the viruses and tracking mutations in their genomes are important for the prevention of AIV spreading and early detection of virus strains with pandemic potential.

The purpose of the investigation was to summarize and analyze results of AI tests carried out by state laboratories during 2020 and the 1st quarter of 2021. The tests were conducted in the framework of state control of AI aimed to early detection and prevention of AIV spreading on the territory of Ukraine.

METHODS

21,891 samples of biological material (4,134 and 17,757 samples from wild and domestic birds, respectively) were tested by PCR. For testing following commercial kits and reagents were used: IndiSpin Pathogen Kit (INDICAL BIOSCIENCE), VetMAX™-Gold AIV Detection Kit (Thermo Fisher Scientific), AIV H5-H7 REAL TIME (ADIAVET), AgPath-ID™ One-Step RT-PCR Reagents (Ambion) with N8 RT-PCR primers and probe (Hoffmann et al., 2016). Positive samples from outbreak in Vinnytsia oblast were submitted to the OIE reference laboratory for AI (APHA, Weybridge) diagnosis confirmation, whole genome sequencing and phylogenetic analysis.

RESULTS

In 2020, 153 positive samples on AI type A (149 samples from poultry and 4 from wild birds) were detected. The presence of AIV RNA subtype H5N8 was confirmed in 84 samples of the poultry of Vinnytsia, Kherson, Kyiv and Mykolaiv oblasts.

In the 1st quarter of 2021, the presence of AIV RNA subtype H5N8 was detected in 61 samples (46 samples from poultry and 15 from wild birds) of Mykolaiv, Kyiv, Donetsk, Ternopil and Kherson oblasts.

In 2020, 9 outbreaks of AI were registered in Vinnytsia (1), Mykolaiv (5), Kyiv (2) and Kherson (1) oblasts; in 2021 (1st quarter) also 9 outbreaks were registered in Mykolaiv (2), Kyiv (2), Donetsk (2), Ternopil (1) and Kherson (2) oblasts.

Bioinformatics analysis of the sequenced genomes of AIVs shows that all isolated viruses had a multi-basic cleavage site motif consistent with a highly pathogenic phenotype (PLREKRRKGLF) and belongs to A/H5 GsGd clade 2.3.4.4b that are currently circulating in Europe.

CONCLUSIONS

During 2020-2021 (1st quarter), 18 outbreaks of AI (3-commercial poultry farms, 12-from backyards and 3-in wild birds) were registered in 6 oblasts of Ukraine. RNA of AIV in the samples from imported birds was not detected. Wild migratory birds are the most likely source of AIV in Ukraine since most outbreaks were registered in oblasts located at the crossing of wild bird migratory routes.
EARLY PROTECTION AGAINST HETEROLOGICAL INFLUENZA INFECTION AFTER INTRanasAL IMMUNIZATION OF MICE WITH LIVE INFLUENZA VACCINE

Topic: Science based management of epidemics and pandemics

Galina Landgraf (1), Yulia Desheva (2)


BACKGROUND
The work studied early protection against heterologous influenza infection after immunization with LHB of the early pandemic subtype A / H7N3 in a mouse model and possible factors of early protection in THP-1 cell culture.

METHODS
Viruses: We used a reassortant vaccine virus A / 17 / Mallard / Netherlands / 00/95 (H7N3), prepared in the Virology Department of the Federal State Budgetary Scientific Institution "IEM", and influenza virus A / South Africa / 3626/13 (H1N1) pdm09. Infection with the A / South Africa / 3626/2013 (H1N1) pdm09 virus (10-50% murine lethal doses, MLD50) was carried out on the 5th and 7th days after immunization with LAIV. Analysis of gene expression of early cytokines in THP-1 cells.

RESULTS
Immunization with LAIV subtype A (H7N3) protected 20% of the immunized animals on the 5th day after immunization and 80% on the 7th day after immunization against lethal infection with the heterologous virus A / South Africa / 3626/2013 (H1N1) pdm09. Revealed a decrease in the reproduction of the infectious virus A / South Africa / 3626/13 (H1N1) pdm09 in the lungs of immunized mice by an average of 10 times.

CONCLUSION
The results obtained indicate that immunization with LAIV partially protected mice against a lethal influenza infection already within the first week after vaccination. This is important for the successful use of LAIV during the period of seasonal rise in morbidity caused not only by influenza viruses, but also by other causative agents of acute respiratory diseases.
The impact of the COVID-19 pandemic on children - the perception of Romanian parents

**Topic:** Societal impact of influenza, RSV disease and COVID-19

Victor Daniel Miron (1,2), Claudiu Filimon (1), Deniz Gunsahin (1), Vlad Alexandru Gaidamut (1), Mihai Craiu (1,2)

1: Carol Davila University of Medicine and Pharmacy, Romania;
2: National Institute for Mother and Child Health "Alessandrescu-Rusescu", Bucharest Romania

**BACKGROUND**

In Romania, as in the rest of the world, the proportion of children confirmed positive for SARS-CoV-2 remains low and severe forms of the disease are exceptional. However, the concern for their children, accompanied by numerous alarming news reports about the pandemic, has led parents to have a disproportionate perception of the impact that COVID-19 may have on the paediatric population. The aim of this paper is to assess the parental perception of the impact of the COVID-19 pandemic on their children.

**METHODS**

We conducted a prospective study based on an anonymous questionnaire that was distributed to parents online via the Virtual Children’s Hospital Facebook pages from August 22-24, 2021. SVC is a virtual medical education space with over 230000 followers and over 210000 likes. A total of 1290 responses were registered, of which 1255 were validated as eligible and included in the final analysis.

**RESULTS**

The majority of respondents were female (95.1%, n=1194), from urban areas (88.8%, n=1114) and had university education (89.6%, n=1124). The mean age of the respondents was 37.6 years (SD ±5.4). Respondents’ fear of not getting COVID-19 decreased significantly from the beginning of the pandemic to the time of the questionnaire, from 79.4% to 24.9% (p<0.001). At the same time the fear of their child/children getting COVID-19 decreased compared to the time of the start of the pandemic, from 76.1% to 48.8% (p<0.001). Only 50.4% consider that wearing the mask protects them from SARS-CoV-2 infection, and 1.3% do not consider wearing the mask useful/necessary. The majority of parents (86.5%) had discussed protection and hygiene rules with their child/children several times. More than a quarter of parents (28.2%) restricted their child/children’s interaction with other children completely during the first 6 months of the pandemic, and 53.9% allowed interaction with a small group of friends/children. In the perception of parents, the closure of schools did not bring major benefits in limiting the COVID-19 pandemic (59.8%), and 77.9% considered that the e-learning system affected their children psychologically and emotionally, but also in terms of the level of knowledge acquired (70.4%).

**CONCLUSIONS**

Fear of COVID-19 has decreased significantly since the onset of the pandemic but remains high among parents for their children. Social isolation measures were perceived by parents as having a negative effect on children. The impact of the pandemic on the pediatric population needs to be monitored and quantified in both short and long term in order to take appropriate action.

**Acknowledgements** All authors had equal contributions. This abstract is part of the license thesis “The impact of the COVID-19 pandemic on the evolution of pediatric pathologies and parental perceptions” performed at the Carol Davila University of Medicine and Pharmacy, Bucharest, Romania. Coordinator: Assoc. Prof. Dr. Mihai Craiu; Supervisor: Dr. Victor Daniel Miron.

**Funding** No funding to declare
Haemagglutinin substitutions N125D, D127E, D222G and R223Q improved replicative fitness and vaccine effectiveness of an A/H1N1pdm09 candidate live attenuated influenza vaccine virus by enhancing α-2,6 receptor binding

**Topic:** Strategies for future Influenza vaccination

Rachael Dempsey (1), Kasia Schewe (1), Lydia Ritter (1), Giulia Tamburrino (2), Annalisa Nucetelli (2), Lauren Parlier (1), Oliver Dibben (1)

1: Flu BPD, Biopharmaceuticals Development & R&D, AstraZeneca, Liverpool, UK;
2: Antibody Discovery and Protein Engineering, Biopharmaceuticals R&D, AstraZeneca, Cambridge, UK

**BACKGROUND**

A/H1N1pdm09 live attenuated influenza vaccine (LAIV) components, A/California/07/2009 and A/Bolivia/S59/2013 (A/BOL13), replicated poorly in primary human nasal epithelial cells (hNEC). This correlated with low-moderate A/H1N1pdm09 vaccine effectiveness (VE) in quadrivalent formulations in vivo and in the outpatient setting. We utilised haemagglutinin (HA) mutagenesis to generate an A/H1N1pdm09 LAIV virus with enhanced replicative fitness in hNEC to replace A/BOL13 in the 2017-18 vaccine formulation, as we hypothesised this would improve VE.

**METHODS**

Site-directed mutagenesis was performed on the egg-propagated wild-type (wt) HA sequence of A/Slovenia/2903/2015 (A/SLOV15), introducing N125D, D127E, D222G and R223Q substitutions in 12 combinations, to generate LAIV variants, S2-S13. In vitro and in vivo characterisation were performed to assess suitability of variants for inclusion in LAIV using hNEC infection, TCID50, haemagglutination-inhibition assays (HAI), HA thermostability, ferret body temperature analyses post-wt challenge, biolayer interferometry (BLI) and in silico modelling.

**RESULTS**

Time-course infection of hNEC demonstrated improved fitness in all variants relative to V1, with S13, carrying all four HA substitutions, producing approximately 1000-fold more virus over five days. Replicative fitness in hNEC was improved without compromising virus yield in eggs for 11 variants, however a ten-fold reduction in titre was observed for the D222G single-substitution variant.

No detectable serum immune response was raised in ferrets for the D127E single-substitution variant when tested by two-way HAI. However, S13, which contained all four substitutions, induced a robust serum immune response and was antigenically-like wt A/SLOV15.

Thermostability data showed that incorporation of N125D, D127E, D222G and R223Q HA substitutions improved thermostability of A/SLOV15 HA protein. S13 lost the ability to agglutinate red blood cells at 60.0°C, relative to 55.0°C for V1.

In vivo data from ferrets showed the four HA substitutions in S13 resulted in LAIV shedding post-vaccination and measurable serum immune responses, relative to no shedding or immune response detected for V1. Post-challenge fever and wt shedding data showed that S13 provided protection from influenza-like illness (ILI) while V1 did not.

BLI receptor-binding assays showed improved binding avidity of S13 to an α-2,6 receptor analogue relative to V1, which showed no detectable binding. This was supported by in silico modelling data, demonstrating an additional interaction in the S13 binding network, which may have contributed to receptor binding differences.

**CONCLUSIONS**

Introduction of N125D, D127E, D222G and R223Q into A/SLOV15 HA protein markedly improved replication in hNEC by increasing α-2,6 receptor-binding avidity, without compromising virus titre in eggs, immunogenicity, antigenicity or HA thermostability. Protection from ILI was confirmed in vivo and A/H1N1pdm09 VE was improved for the 2017-18 LAIV formulation.

This approach was successfully applied during strain development for the 2021-22 LAIV formulation. The four substitutions were introduced into A/Victoria/1/2020 HA protein, improving hNEC replication by approximately 1000-fold, without compromising egg yield, HA thermostability, immunogenicity or antigenicity.
**OVX836, NP-BASED UNIVERSAL INFLUENZA VACCINE CANDIDATE: RESULTS OF PHASE 2A CLINICAL TRIAL**

**Topic:** Strategies for future Influenza vaccination

Isabel Leroux-Roels (1), Jessika Tourneur (2), Geert Leroux-Roels (1), Delphine Guyon-Gellin (2), Alexandre Le Vert (2), Florence Nicolas (2), Paul Willems (2)

1: Ghent University, Ghent, Belgium; 2: OSIVAX, Lyon, France

**INTRODUCTION**

Cellular immunity to well-conserved influenza nucleoprotein (NP) is associated with protection against influenza disease, providing strong rationale for NP-based influenza universal vaccine. OVX836 is an unadjuvanted recombinant vaccine composed of the NP sequence of Influenza A virus fused to Oligodom™, OSIVAX’s proprietary pro-immunogenic tag. We have previously shown that OVX836 induces NP-specific CD4+, CD8+ T-cell and IgG responses in mice, protects mice and ferrets from challenges with multiple strains of influenza, and is safe and immunogenic in humans (Phase 1 trial).

**METHOD**

Randomized, double-blind, reference-controlled Phase 2a study evaluating immunogenicity and safety of one intramuscular dose of OVX836 at 90µg and 180µg in healthy 18-65 year-old subjects, compared to Influvac Tetra™, quadrivalent seasonal influenza sub-unit vaccine.

**RESULT**

**Immunogenicity:** Primary immunogenicity endpoint achieved (superiority of OVX836 over Influvac Tetra in terms of change Day0–Day14 of NP-specific T-cell IFN-γ activity by ELISPOT); median D0/D1 ratio 1.83, 1.63 and 1.03 for OVX836 90µg, OVX836 180µg, Influvac Tetra, respectively (overall difference, p=0.0006). Data support higher immunogenicity for 180µg than 90µg dose: (i) on D8 and D29 and for NP-specific CD4 T-cell responses, OVX836 180µg was significantly different from OVX836 90µg (p=0.0406 and p=0.0353, respectively); (ii) for total T-cells and humoral (anti-NP IgG) response, there was a trend for a dose-effect at D8 and D29.

**Safety:** Both dosages of OVX836 are safe and well-tolerated, comparable to Influvac Tetra. Low incidence of “severe” events, similar to Influvac Tetra and no dose-limiting effects even for the 180µg dose.

**Efficacy:** Observed numbers of influenza-like illnesses (ILI) occurring during influenza season as of 14 days post-vaccination were 8, 2 and 3 in OVX836 90µg, OVX836 180µg and Influvac Tetra groups respectively, suggesting a potential protective effect of OVX836 at 180µg.

**CONCLUSION**

OVX836 was immunogenic with 180µg being superior to 90µg, while both doses were safe and well-tolerated. A signal for efficacy was observed at the 180 µg dose.
Estimation of reduction in influenza vaccine effectiveness due to egg-adaptation changes: systematic literature review and expert consensus

**Topic:** Strategies for future Influenza vaccination

**Emanuele Montemoli (1), Raul Ortiz de Lejarazu (2), Radek Wojcik (3), Solomon Christopher (3), Ilene Panani (4), Antoni Trilla Garcia (6), Helmut Fickenscher (7), Barbara Gaertner (8), Ravi Jandhyala (3), Maria Zambon (9), Catherine Moore (10)**

1: Department of Molecular Medicine, University of Siena, Italy; 2: Valladolid National Influenza Centre, Valladolid, Spain; 3: Medialis Ltd, Banbury, United Kingdom; 4: Open Rome, France; 5: Department of Biomedical Science for Health, University of Milan, Italy; 6: Preventive Medicine and Epidemiology, Hospital Clinic, University of Barcelona, Spain; 7: Institute for Infection Medicine, Kiel University, Germany; 8: Saarland Medical University, Germany; 9: Public Health England, United Kingdom; 10: Wales Specialist Virology Centre, Public Health Wales, United Kingdom

**BACKGROUND**

Influenza vaccines are the main tool to prevent morbidity and mortality resulting from influenza infection. Egg-adaptation changes associated with the egg-based manufacturing process may reduce vaccine effectiveness. At the time of this study, no data was available on the extent of this impact. This study aimed to provide the first estimate of the impact that egg-adaptation changes and antigenic drift have on the effectiveness of trivalent influenza vaccines (TIV) and quadrivalent influenza vaccines (QIV).

**METHODS**

Eleven leading European experts in influenza (virologists) were recruited to the study and nine completed a Delphi-style exercise between July and December 2020. In the first round, the experts were asked to answer open-ended questions on the impact of antigenic drift and egg-adaptations on vaccine match (VM) and influenza vaccine effectiveness (IVE), and its frequency. In parallel, a SLR was conducted, reviewing IVE and VM data for the 2014-2019 influenza seasons, as well as mapping the occurrence of antigenic drift and egg-adaptation changes in these seasons. In the second round, the experts were presented with the data from the SLR and aggregated experts’ responses to round one questions. These were compiled at the European level and by country. The experts were asked to review and confirm or amend their responses in the second round, after which the estimates were finalised.

**RESULTS**

The experts estimated that across Europe, egg-adaptation changes reduced TIV and QIV match on average by 18% (all strains and age groups) and effectiveness by 9% (all strains and age groups, 2014-2019 seasons). According to the experts, antigenic drift – traditionally considered one of the key reasons for influenza vaccine ineffectiveness, results in a similar, however slightly higher, impact on VM (8-24%) and IVE (5-20%). This highlights the importance egg-adaptation changes have on IVE. The highest reduction in IVE was estimated for the influenza virus A(H3N2) subtype for the under 65 age group (up to 16%). When asked about the frequency of the phenomena, the experts indicated that, on average, between 2014-19 influenza seasons, egg-adaptation changes and antigenic drift significant enough to impact IVE occurred in two and three out of five seasons, respectively. They also agreed that this pattern is likely to re-occur in the following seasons.

**CONCLUSION**

Expert estimates suggest there is a potential for 9% on average and up to a 16% increase in IVE (against the A(H3N2), <65 age group) if egg-adaptation changes that arise when employing the traditional egg-based manufacturing process are avoided. The selection of alternative technologies for vaccine production may, thus, be an important step in the development of immunisation strategies.
A Prospective Cohort Study on Pregnancy Outcomes in Women Immunized with Seasonal Cell Culture-Derived Quadrivalent Influenza Vaccine (QIVc) During Pregnancy

**Topic:** Strategies for future influenza vaccination

**Authors:** Josephine van Boxmeer (6), Jessica Albano (2), Hugh Tilson (3), Anthony Szilassi (6), John Vanchiere (5), Ellis Ikes (6), Daphne Sawlwin (7), Deborah Malinine (1), Matthew Hoekenboken (2), Jonathan Edelman (8), Christopher Robinson (9)

1. Seqirus - a CSL Company, Cambridge, MA, United States of America;
2. Syneos Health, Wilmington, NC, United States of America;
3. UNC Gillings School of Global Public Health, Chapel Hill, NC, United States of America;
4. Scally Consulting LLC, Washington DC, United States of America;
5. LSU Health Science Center, Shreveport, LA, United States of America;
6. Seqirus Netherlands B.V., Amsterdam, the Netherlands;
7. Seqirus Australia Pty Ltd, Parkville, VIC, Australia;
8. Seqirus USA Inc., Summit, NJ, United States of America;
9. Charleston Maternal Fetal Medicine, Mount Pleasant, SC, United States of America

**OBJECTIVES**
A pregnancy exposure registry was established to fulfill a US Food and Drug Administration commitment for a postmarketing safety study of QIVc (FLUCELVAX®). This population-based prospective cohort study collected data on pregnancy outcomes and events of interest reported from women immunized with QIVc during pregnancy. To overcome the challenges with spontaneous enrollment presented from similar studies, a targeted approach was implemented using OB/GYN clinics utilizing QIVc as part of routine care.

**METHODS**
A prospective observational cohort study was conducted that enrolled pregnant women immunized with QIVc as part of routine care. Women could participate in the study following vaccination in any trimester and prior to pregnancy outcome. During the first influenza season, the study relied on spontaneous enrollment by healthcare providers or self-enrollment of pregnant women. In the second and third season, study data were provided by OB/GYN clinics that were identified using QIVc in their seasonal influenza vaccination campaigns during routine prenatal care. Primary safety assessments included occurrence of adverse pregnancy outcomes and infant events of interest of preterm birth (PTB), low birthweight (LBW) and Major Congenital Malformations (MCM). A teratologist reviewed all reported malformations and classified them using the Center for Disease Control and Prevention (CDC) code of the MCM and other outcomes of interest.

**RESULTS**
This study enrolled 693 women who had received QIVc as part of routine care over the course of three Northern Hemisphere influenza seasons (2017-2020). Of these, 665 women were evaluable and 27 (3.9%) were lost to follow-up. The study population was diverse and key risk groups for adverse pregnancy outcomes were represented. In season 1 (2017-2018), the enrollment rate was low at 5 subjects/year. When the OB/GYN clinic-based approach was implemented in season 2, resulting in 5 participating clinics, the enrollment rate increased to approximately 325 subjects/year. For pregnancy and infant outcomes, >99% (95% CI: 98.0%-99.7%) of pregnancies resulted in live birth. For PTB a prevalence of 5.2% (upper 95% CI: 11.5%) was observed, for LBW a prevalence of 5.8% (upper 95% CI: 7.6%) was observed, and for MCMs according to CDC MCDP criteria a prevalence of 1.9% (upper 95% CI: 3.1%) was observed. The point estimates of the prevalence observed in the study population was below the prevalence reported in background data for the general US population.

**CONCLUSIONS**


Budget Impact Analysis of the MF59-Adjuvanted Quadrivalent Influenza Vaccine in the Older Adult French Population

Topic: Strategies for future Influenza vaccination

Van H Nguyen (1), Paolo D’Agostino (2), Nathalie Phalippon (3), Andrea McCracken (4), Joaquin Mould-Quevedo (5)

1: VH Consulting, Montreal, Canada;
2: Seqirus GmbH, Munich Germany;
3: Laboratoire Arrow, Lyon France;
4: Seqirus UK Limited, Maidenhead UK;
5: Seqirus USA Inc., Summit, NJ, United States of America

BACKGROUND
Influenza is a highly contagious, viral respiratory disease that can cause severe illness and fatal complications. However, the risk of hospitalization and death due to influenza is strongly age-related and is highest among older adults. Immunosenescence, an age-related functional decline in the immune system, plays an important role reducing immune competence against infections and increasing the susceptibility of older adults to severe disease and complications of influenza. The aim of this study was to evaluate the budget impact of a national immunization program based on the progressive use of the MF59-Adjuvanted Quadriivalent Influenza Vaccine (aQIV) versus the current scenario of vaccinating with standard egg-based quadrivalent influenza vaccines (QIVe) or an inactivated high-dose quadrivalent influenza vaccine (QIV-HD) in French older adults (aged 65yrs+). Analyses are made from the payer perspective.

METHODS
A static decision-tree model with French demography was used to provide realistic estimates of the impact of aQIV penetration on age-specific influenza infections (65-74yrs and 75yrs+). The model forecasts influenza-related costs and benefits for the next 3 seasons (2021-2022 as the base year). Relative vaccine effectiveness of aQIV and QIV-HD over QIVe for subjects 65yrs+ of age were assumed from published Real World Evidence studies and one Randomized Clinical Trial, respectively. Influenza-related probabilities of medical care visit, outpatient complications, hospitalization, mortality, and direct costs were extracted from published literature in France. The budget impact model includes a moderate increasing penetration of aQIV over the next 3 years in the French market (primary taking market share from QIVe), using a similar local price per dose to QIV-HD (30.25 euros). Deterministic sensitivity analyses were conducted.

RESULTS
Over the 3-year period, switching gradually from QIVe to aQIV, would result in both significant health benefits and complications net budget savings. Although the progressive introduction of aQIV produces higher vaccination costs of about €105.3M (+27.9%), the total savings derived from fewer influenza events and complications resulted in an overall cost increment of €80.9M (6.7%). Budget savings were mainly driven by the avoidance of medical care visits costs (€670K); outpatient complication costs (€788K) and inpatient complication costs (€23.2M). Replacing gradually QIVe with aQIV in French older adults’ subjects can, over a 3-year period, prevent 56,028 influenza cases, 13,449 medical care visits, 30,815 outpatient complications, 3,902 inpatient complications, and 745 influenza-associated deaths.

CONCLUSIONS
Based on the economic model, the progressive introduction in France of aQIV, MF59-Adjuvanted Quadrivalent Influenza Vaccine, would be clinically favorable to the National immunization program while the incremental overall cost impact may be small.
Strategies for future Influenza vaccination

An Economic Evaluation of Enhanced Influenza vaccines for the Elderly in Spain. The Adjuvanted Quadrivalent Influenza Vaccine versus High-Dose Quadrivalent Influenza Vaccine

**Topic:** Strategies for future Influenza vaccination

**Sergio Marquez Pelaez (1), Ray Gani (2), Piedad Alvarez (2), Richard Guerrero-Ludueña (2), Jesus Ruiz-Aragon (3)**

1: Universidad Pablo de Olavide, Sevilla, Spain; 2: Evidera, London, England; 3: Hospital Universitario Puerta del Mar, Cádiz, Spain

**BACKGROUND**

Adults 65 years of age or older are particularly vulnerable to the complications resulting from influenza infections with higher risk of severe health outcomes than younger people. Vaccine efficacy has been estimated to be 60% in adults aged 18–65 years. However, several studies have reported a reduction in vaccine effectiveness in older adults that could be explained by immunosenescence. Thus, enhanced influenza vaccines have been designed in the elderly population to provide improved immune response. The objective of this study is to evaluate the cost-effectiveness of two enhanced vaccines, the adjuvanted quadrivalent influenza vaccine (aQIV) versus the high-dose quadrivalent influenza vaccine (HD-QIV) in the elderly (65yrs+), from the payer and societal perspective in Spain.

**METHODS**

A decision-tree model with Spanish demography was used to provide realistic estimates of the impact of aQIV and HD-QIV on 65yrs+ influenza infections. The model was calibrated to forecast influenza-related costs and benefits for a one-year time-horizon in Spain considering official available local data. A small absolute benefit, non-statistically significant, for the relative vaccine effectiveness (rVE) of aQIV over HD-QIV was assumed similar to recent published meta-analysis outcomes. Influenza-related probabilities of outpatient visit, hospitalization, work absenteeism, mortality, with associated (dis)utilities and costs were extracted from Spanish and European published literature. Official Spanish tender prices for aQIV (€13) and HD-QIV (€25) were considered in the analysis. Deterministic and probabilistic sensitivity analyses (PSA) were conducted.

**RESULTS**

Using aQIV instead of HD-QIV in older adults can annually prevent 4,141 symptomatic Influenza cases, 759 office visits, 441 hospitalizations and 26 deaths. The annual number of quality-adjusted life-years increase by 218 and yield cost-saving from the payer perspective (€63.5 M savings) and the societal perspective (over €100.0 M savings). PSA confirmed base case outcomes robustness.

**CONCLUSIONS**

If aQIV is selected for the Spanish immunization program, it is expected to lead to a significant reduction in the number of outpatient visits and hospitalizations in the elderly and would allow substantial savings in comparison to HD-QIV.
Developing a patient-centered digital intervention for increasing influenza vaccination rates in people with cardiovascular disease using a remote mixed-methods approach

**Topic:** Strategies for future Influenza vaccination

Jessica Schreeder (1), Mohammad Madjid (2), Mrudula R. Munagala (3), John Piette (4), L.J. Tan (5), Orly Vardeny (6), Jan Liska (7), Jennifer L. Lee (1,8), Wei-Yi Chih Lee (1), Neil Marshall (2), Monica Mercer (9), Julia Ryan (1,10), Sandrine Samson (11)

1: Evidation Health, San Mateo, CA, USA;  
2: The University of Texas Health Science Center, McGovern Medical School, Houston, TX, USA;  
3: University of Miami, Miller School of Medicine, Miami, FL, USA;  
4: University of Michigan, Ann Arbor, MI, USA;  
5: ImmunoGenetics Action Coalition, St. Paul, MN, USA;  
6: Center for Care Delivery and Outcomes Research, VA Health Care System, Minneapolis, MN, USA;  
7: Sanofi, Gentilly, France;  
8: Emory University, Atlanta, GA, USA;  
9: Sanofi Pasteur, Swiftwater, PA, USA;  
10: University of California-Berkeley, Berkeley, CA, USA;  
11: Sanofi Pasteur, Lyon, France

**BACKGROUND**

Influenza vaccination rates for individuals with cardiovascular diseases (CVD) are sub-optimal, despite risks for poor outcomes with influenza, like heart attack and stroke. Digital interventions can increase influenza vaccination rates, but require knowledge of complex individual and systemic barriers. Patient Centered Outcomes Research Institute (PCORI) recommends incorporating patient perspectives in interventions, but implementation of human-centered methods remains limited. This study aims to describe barriers and drivers to influenza vaccination in people with CVD, and the process of developing a patient-centered intervention designed to increase influenza vaccination rates.

**METHODS**

This mixed-methods study included adults in the United States who self-reported having CVD. Between May-August 2020, participants completed an online survey (n=844) focused on health/vaccination history, barriers/beliefs around vaccination, and knowledge of health risks via a health platform (Achievement). Descriptive statistics and correlations were conducted. A subset of 23 participants with inconsistent vaccination history completed virtual semi-structured interviews exploring perceptions around vaccination and reactions to message themes and designs. Messages were developed using survey data and research, and iteratively refined throughout the interviews.

**RESULTS**

Survey participants were predominantly female (68%) and white (86%), with an average age of 48.9 (SD: 12.8). Nearly 82% of participants reported having at least some college education. On average, 50.6% of participants reported getting the influenza vaccine every year for the previous 5 years, while 17.9% reported no influenza vaccination in the previous 5 years. Most influential facilitator of vaccination was healthcare provider recommendation to vaccinate. Barriers included inconvenience and distrust of vaccines. Approximately 40% of participants were unaware/unsure if people with CVD were a high-risk group. Participants reported uncertainty surrounding the impact of COVID-19 on their influenza vaccination plans. Qualitative data identified additional misconceptions, like believing that the vaccine can cause influenza. Participants thought successful messages would need to be engaging, reference trustworthy sources, and be understandable.

**CONCLUSIONS**

This online-executed mixed-methods study identified beliefs underlying decision-making about influenza vaccination in a high-risk population. Results documented the range of factors that influence vaccination decisions, identified unique beliefs regarding vaccination in people with CVD, and informed the development of messages using the patient’s voice (see figure below). Message effectiveness will be determined via randomized control trial. Mixed-methods approaches can be used in technologically-engaged communities to develop public health messages that resonate with the larger community and possibly influence change in vaccination behavior, particularly in rapidly-evolving situations, such as with novel viruses and vaccines.

Sanofi Pasteur funded this study.

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**Figure 1. Flow for targeted Multi-Media Intervention messages**
Preclinical ferret study of universal live-attenuated influenza vaccine candidates expressing multiple M2e epitopes

**Topic:** Strategies for future Influenza vaccination

Daria Mezhenskaya (1), Irina Isakova-Sivak (1), Victoria Matyushenko (1), Svetlana Donina (1), Andrey Rekstin (1), Konstantin Sivak (2), Kirill Yakovlev (2), Anastasia Katelnikova (3), Kirill Kryshen (3), Valery Makarov (3), Larisa Rudenko (1)

1: Institute of Experimental Medicine, Russian Federation; 2: Smorodintsev Research Institute of Influenza, Russian Federation; 3: Institute of Preclinical Research Ltd., Russian Federation

**BACKGROUND**
The development of a universal influenza vaccine with a wide spectrum of protection and durability is a serious public health problem. Highly conserved extracellular domain of matrix 2 protein (M2e) is an attractive target for the induction of cross-protective immune response. Here, we studied two recombinant live attenuated influenza vaccines (LAIVs) expressing additional four M2e-epitopes within HA (LAIV/HA+M2e) or NS (LAIV/NS+M2e) molecules.

**METHODS**
Male ferrets were immunized intranasally with two doses of either LAIV/HA+M2e or LAIV/NS+M2e recombinant viruses, or with the H3N2 LAIV control virus. Shedding of the LAIV viruses was determined by titration of nasal wash samples collected during 4 days after each vaccination in eggs. Safety of vaccines was monitored by the clinical signs of infection during the course of immunization. Immune response was determined using a standard ELISA against H3N2 whole virus and against M2e recombinant protein. Protective effect of the vaccines was assessed by infecting immunized ferrets with pandemic H1N1 virus. An indirect protection of immune sera was assessed in mice against a panel of PR8-based viruses encoding M genes of different origin. In addition, the levels of IgG antibody secreted by mediastinal lymph nodes (MLN) collected 5 days after challenge were measured.

**RESULTS**
All LAIVs showed high level of replication after the first immunization and no signs of viral shedding after the second dose. No signs of disease were detected during the immunization phase. All LAIVs induced high levels of anti-H3N2 IgG antibodies, whereas only recombinant vaccines induced significant level of anti-M2e IgGs with notable boost effect. Of note, LAIV/HA+M2e resulted in higher anti-M2e antibody titers, compared to LAIV/NS+M2e vaccine. Further challenge study revealed correlation between the rate of protection and the level of anti-M2e antibodies. Ferrets vaccinated with LAIV/HA+M2e showed less clinical symptoms after challenge and had lower viral shedding in respiratory tissues. Mouse challenge study confirmed that the M2e-based vaccines had higher protective effect than the control LAIV virus. In vitro stimulation of MLN cells revealed that the M2e-specific antibody most probably contributed to the overall protection, as the magnitude of M2e-binding antibody responses was the highest in the LAIV/HA+M2e-immunized ferrets.

**CONCLUSIONS**
Preclinical study of universal live-attenuated influenza vaccines in ferrets proved that all vaccines were safe and immunogenic. The LAIV/HA+M2e vaccine prototype had the highest ability to induce anti-M2e antibodies and therefore had higher rate of protection. This candidate warrants its further evaluation in a phase 1 clinical trial.

**Funding** The research was funded by RSF grant №19-15-00015.
Strategies for future Influenza vaccination

Functional serum antibody induced by inactivated influenza vaccines as correlate of protection and predictor of the efficacy benefit of new vaccine formulations. A review in anticipation of mRNA vaccines.

Topic: Strategies for future Influenza vaccination

Walter Emil Philipp Beyer (1), Bram Palache (2), Mimoun Boulouch (3), Albert Osterhaus (4)

1: Erasmus MC Rotterdam, The Netherlands; 2: FluPal Consultancy, Amstelveen, The Netherlands; 3: University of Amsterdam, The Netherlands; 4: University of Veterinary Medicine, Hannover, Germany

BACKGROUND

Vaccine efficacy in preventing influenza A and B virus infection should ideally be estimated by randomised placebo-controlled field trials with laboratory-confirmed influenza disease as primary outcome. Such trials are laborious, time-consuming, unethical in persons at risk for influenza-related complications, and their success depends on sheer luck, namely the unpredictable extent of virus circulation during the influenza season following vaccination. For mRNA influenza vaccines in development, immunogenicity trials will be a more practicable alternative with functional serum antibody (FSAb) titre serving as surrogate variable for field protection. To identify advantages and pitfalls of this approach, it may be fruitful to review the longstanding clinical experience with FSAb immunogenicity trials comparing inactivated or live-attenuated influenza vaccines, on the following questions:

- Haemagglutination inhibition (HI) or microneutralisation (MN), which FSAb assay should be preferred?
- How strong is the association between FSAb and clinical protection?
- How to predict field vaccine efficacy from post-vaccination FSAb titres?
- What is a meaningful superiority threshold for the ratio between post-vaccination geometric mean titres when comparing two vaccine formulations?

METHODS

Review of the latest studies on FSab assays, and immunogenicity and field trials with influenza vaccines, and meta-analyses of such trials.

RESULTS

Biologically, neutralization of viral attachment to cells and consecutive cell infection is more plausible than inhibition of viral attachment to cells alone. Dependency of the HI assay from erythrocytes is an increasing source of operational problems. Alternative MN assays are available, and their broader use is advocated.

The latest systematic review for inactivated influenza vaccine (2010) revealed a statistically strong association between FSAb and clinical protection, allowing the estimation of a non-linear protection curve. A number of large-scale randomized field trials since 2010 confirmed the association. For live-attenuated influenza vaccine, the association between FSAb and clinical protection is not strong because a relevant part of vaccine-induced antibody (secretory IgA) is not captured by FSaB assays. Dichotomous response variables based on seroprotection or seroconversion are often insufficiently expressing clinical protection. When using the continuous variable of post-vaccination FSAb titre and linking it to the above mentioned protection curve, field vaccine efficacy can be reliably predicted when priming history is taken into account. Based on extensive experience with adjuvanted vaccines, a superiority threshold of 1.5 is suggested, i.e. a given vaccine formulation is regarded superior to a comparator formulation when it induces, on average, 1.5-fold larger post-vaccination antibody titres than comparator.

CONCLUSIONS

Immunogenicity trials assessing functional serum antibody, preferably by a microneutralization assay, are likely to be a good predictor of the field efficacy of mRNA vaccines and may be used to establish similarity with, or even superiority to inactivated influenza vaccines.
Quadrivalent M2SR (M2-deficient Single Replication) Live Influenza Investigational Vaccine Protects Ferrets Against Drifted Influenza B Virus Challenge

**Topic:** Strategies for future Influenza vaccination

Lindsay Hill-Batorski, Yasuko Hatta, Michael Moser, David Marshall, Pamuk Bilsel

FluGen, Inc., Madison, WI, USA

**BACKGROUND**

Quadrivalent inactivated influenza vaccines (QIV) induce neutralizing antibodies against viral hemagglutinin (HA). Despite annual update of HA vaccine antigens, current vaccines provide ~60% vaccine effectiveness (VE). QIV VE can be as low as 10% when circulating strains do not match vaccine HA. The live M2SR (M2-deficient single replication) influenza vaccine candidate has shown broad humoral, mucosal and cellular immune responses and protection against multiple influenza A subtypes. Here we show similar properties with Quadrivalent M2SR against drifted influenza B challenge compared to QIV.

**METHODS**

Ferrets pre-infected with influenza H1N1 and B/Yamagata viruses were immunized intranasally with PBS-Mock or Quadrivalent M2SR, or intramuscularly with FluZone QIV. Serum collected post-vaccination was evaluated for antibody responses. Forty-two days post-vaccination, ferrets were challenged intranasally with 10^6 pfu of B/Brisbane/60/2008 (Victoria) influenza virus. Nasal washes, nasal turbinate, trachea, and lungs were taken post-challenge and evaluated for virus by TCID50 assay.

**RESULTS**

Quadrivalent M2SR and QIV elicited high serum antibodies against the vaccine strain B/Colorado/06/2017 (Fig. 1A) and against the drifted influenza B challenge strain B/Brisbane/60/2008 (Fig. 1B) in ferrets with preexisting immunity. Like Mock, ferrets who received QIV displayed both weight loss (6.2%, Fig. 2A) and a rise in temperature (1.1°C, Fig. 2B) after challenge. In contrast, the Quadrivalent M2SR group did not exhibit any significant weight or temperature changes after challenge. Quadrivalent M2SR controlled the drifted challenge virus better than QIV as evidenced by significantly lower or absent post-challenge virus titer in nasal washes (Fig. 3A) and nasal turbinate (Fig. 3B).

**CONCLUSION**

Despite eliciting similar antibody titer sizes, the Quadrivalent M2SR demonstrated superior protection compared to QIV in a drifted influenza B challenge model in ferrets. These results suggest that the intranasal M2SR platform may confer additional advantages over current vaccines. Quadrivalent M2SR is in late-stage development for testing in a first-in-human clinical study.

![Figure 1: Plaque reduction neutralization test (PRNT) antibody titers for Quad M2SR and QIV against matched Influenza B vaccine strain B/Colorado/06/2017 (Fig. 1A) and drifted strain B/Brisbane/60/2008 (Fig. 1B) on pre-study (Day -3), pre-vaccination (Day 28), and 3 weeks post-vaccination (Day 51). The detection limit of the assay (horizontal dashed line) was 15 PRNT50.](image-url)
Figure 2. Percent body weight changes (Fig. 2A) and average body temperatures changes (Fig. 2B) following challenge with drifted Influenza B strain B/Brisbane/60/2008 for ferrets vaccinated with Quad M2SR or QIV.

Figure 3. Viral titers in nasal washes (Fig. 3A) and nasal turbinates (Fig. 3B) collected post-challenge with Influenza B strain B/Brisbane/60/2008 in ferrets vaccinated with Quad M2SR or QIV. No virus was detected in the trachea or lungs. The detection limit of the assay (horizontal dashed line) was 1.5 log10 TCID50/mL and 20 FFU respectively. For Nasal washes, a one-way analysis of variance (ANOVA) was used to compare groups and found to be significant (p value < 0.0001). This was followed up with Multiple t tests to compare between groups #p<0.05, *p<0.01, **p<0.0001.
Importance and Value of Adjuvanted Influenza Vaccine in the Care of Older Adults from a European Perspective – A Systematic Review of Recently Published Literature on Real-World Data

**Topic:** Strategies for future Influenza vaccination

Barbara C. Gärtner (1), Thomas Weinke (2), Klaus Wahle (3), Anja Kwetkat (4), Dietmar Beier (5), Kim J. Schmidt (6), Tino F. Schwarz (7)

1: Institute of Medical Microbiology and Hygiene, Saarland University Hospital and Medical Faculty of Saarland University, Homburg/Saar, Germany;
2: Clinic of Gastroenterology and Hepatology, Ernst von Bergmann Klinikum, Potsdam, Germany;
3: University of Münster, Münster, Germany;
4: Department of Geriatrics, Jena University Hospital, Jena, Germany;
5: Member of Saxon Committee on Vaccinations (SIKO), Chemnitz, Germany;
6: Clinic of Gastroenterology and Infectiology, Ernst von Bergmann Klinikum, Potsdam, Germany;
7: Institute of Laboratory Medicine and Vaccination Centre, Klinikum Würzburg Mitte, Würzburg, Germany

**BACKGROUND**

There is an urgent need for improved influenza vaccines especially for older adults due to the presence of immunosenescence. It is therefore highly relevant to compare enhanced influenza vaccines with conventional influenza vaccines regarding their effectiveness. The European Centre for Disease Prevention and Control (ECDC) assessed the evidence on efficacy, effectiveness and safety of enhanced influenza vaccines vs. conventional influenza vaccines available until 7 February 2020. The review identified 12 effectiveness studies comparing MF59-adjuvanted vaccine with conventional vaccines regarding influenza-related outcomes. Evidence on effectiveness of adjuvanted vs. conventional vaccines was considered uncertain, while efficacy and effectiveness data for high-dose vaccine vs. conventional vaccines suggested a better protection with the enhanced vaccine. The objective of our study was to assess the evidence for adjuvanted trivalent and quadrivalent influenza vaccines (aTIV/aQIV) vs. non-adjuvanted trivalent and quadrivalent standard-dose (TIV/QIV) and high-dose (TIV-HD/QV-HD) influenza vaccines regarding influenza-related outcomes in older adults, which has become available since ECDC’s review.

**METHODS**

A systematic literature search was conducted in Embase and MEDLINE to identify randomised controlled trials, observational studies and systematic reviews, published since ECDC’s systematic review of enhanced seasonal influenza vaccines (between 7 February 2020 and 6 September 2021) and assessing efficacy/relative vaccine effectiveness (rVE) in adults aged ≥60 years. Included studies were appraised with either the Cochrane Risk of Bias tool, ROBINS-I or AMSTAR 2.

**RESULTS**

Eleven analyses from nine RWE studies comprising ~53 million participants and assessing the rVE of aTIV vs. TIV, QIV and/or TIV-HD in adults aged ≥65 years over the 2006/07-2008/09 and 2011/12-2019/20 influenza seasons were identified. Nine analyses found that aTIV was more effective than TIV and QIV in reducing suspected influenza outbreaks and influenza-related medical consultations by clinical setting (rVE ranging from 7.5% to 25.6% for aTIV vs. TIV and 7.1% to 36.3% for aTIV vs. QIV). Seven analyses found similar effectiveness of aTIV vs. TIV-HD in reducing influenza-related medical encounters, inpatient stays and hospitalisations/emergency room visits. In three analyses, aTIV was significantly more effective than TIV-HD in reducing influenza-related medical encounters and office visits (rVE ranging from 6.6% to 16.6%). Risk of bias of identified studies was moderate to high.

**CONCLUSIONS**

In addition to the 12 effectiveness studies identified in ECDC’s review, our study identified eight studies assessing the rVE of aTIV vs. TIV/QIV and seven studies assessing the rVE of aTIV vs. TIV-HD, published between 7 February 2020 and 6 September 2021 and comprising ~53 million older adults. aTIV was found to be more effective than TIV and QIV and similar in effectiveness as TIV-HD in reducing risk of influenza-related outcomes. Our study suggests that both adjuvanted and high-dose vaccines are effective alternatives for vaccination programmes in older adults and preferable over conventional standard-dose vaccines.
Correlation Between Patient-reported and Clinician-assessed RSV Symptoms and Case Definition to Capture Moderate-to-Severe RSV Disease in Adults Aged ≥65 Years: A Randomized, Placebo-controlled, Phase 2b Study

**Topic:** Strategies for future RSV disease vaccination

Ann R. Falsey (1), Kristi Williams (2), Efthimia Gymnopoulou (3), Stephen Bart (4), John Ervin (5), Arangassery Rosemary Bastian (6), Joris Menten (3), Els De Paepe (3), Hilde de Boer (7), Sjoeka Vandenberge (8), Eric Chan (8), Jared Sadoff (6), Macaya Douoguih (6), Benoît Callendret (6), Christy A. Comeaux (6), Esther Heijnen (6)


**BACKGROUND**

Respiratory syncytial virus (RSV) can cause serious lower respiratory tract disease (LRTD) in older adults. In a Phase 2b proof-of-concept trial of an Ad26.RSV.preF-based vaccine for the prevention of RSV-mediated LRTD in adults aged ≥65 years (CYPRESS), exploratory analyses evaluated correlations between patient-reported and clinician-assessed acute respiratory infection (ARI) signs/symptoms and evaluated a case definition for LRTD.

**METHODS**

CYPRESS (NCT03982193) is a randomized, double-blind, placebo-controlled Phase 2b trial. Adults aged ≥65 years were randomized 1:1 prior to the RSV season to receive vaccine or placebo. The primary endpoint was the first occurrence of RT-PCR-confirmed RSV-mediated LRTD during the RSV season according to any of 3 case definitions based on new onset or worsening of lower respiratory tract infection (LRTI) symptoms (cough, shortness of breath, sputum production, wheezing) with or without systemic symptoms (fatigue, fever). During an ARI episode, patient-reported signs/symptoms were collected daily using an eDiary that included the RSV-specific Respiratory Infection Intensity and Impact Questionnaire (RiiQ; each symptom rated from 0 = none to 3 = severe), and clinician-assessed signs/symptoms (each symptom rated from 0 = no symptoms to 3 = bothersome most of the time) were collected on Day 3, 4, or 5. Correlations between patient-reported and clinician-assessed symptoms were calculated by polychoric (symptoms rated on a 0-3 scale) and tetrachoric (symptoms rated as present/absent) correlations. The correlation between a selected LRTD case definition and evaluated moderate/severe RSV-mediated LRTD in adults aged ≥65 years (CYPRESS), exploratory analyses evaluated correlations between patient-reported and clinician-assessed acute respiratory infection (ARI) signs/symptoms and evaluated a case definition for LRTD.

**RESULTS**

A total of 5782 participants (2891 in each study arm) received study treatment. Vaccine efficacy was 65.8–80.0% depending on case definition. Clinician assessment and RiiQ scores from the same day were strongly correlated. Polychoric and tetrachoric correlations were, respectively, 0.76 and 0.89 for cough, 0.76 and 0.78 for shortness-of-breath, 0.65 and 0.77 for sputum production, and 0.69 and 0.80 for fatigue. Polychoric correlation was 0.74 for wheezing. Of 96 participants with any RSV ARI, the CEC assessed 3 as severe, 30 as moderate, 7 as mild, and 16 as not LRTI; all severe, 26/30 moderate, and 5/7 mild LRTIs met the selected LRTD case definition versus 2/16 of those with no LRTI. In the placebo arm, the selected LRTD case definition captured RSV-associated hospitalizations (1/1), complications (7/7), clinically relevant disease (3/3), therapeutic interventions (8/8), and emerging MRU (9/10).

**CONCLUSIONS**

In CYPRESS, patient-reported RiiQ scores were strongly correlated with clinician-assessed symptoms. An LRTD case definition of new onset or worsening of ≥3 symptoms of LRTI captured most CEC-classified moderate-to-severe RSV-mediated LRTD, as well as most events associated with other indicators of moderate-to-severe RSV disease.

**Study Group CYPRESS**

**Keywords:** Respiratory syncytial virus, viral vaccines, adult immunization, patient-reported outcomes, lower respiratory tract disease

**Category/Section:** Scientific programme – Strategies for future influenza and RSV disease vaccination
How to encourage people to get vaccinated against COVID-19? The role of self-persuasion targeted at others' health and empathy among young adults

Topic: Strategies for future RSV disease vaccination

Dariusz Drążkowski, Radosław Trepanowski, Patrycja Chwikowska, Magda Majewska

Adam Mickiewicz University, Poland

BACKGROUND
Persuading people to vaccinate is crucial in fighting COVID-19. Encouraging young people to vaccinate is difficult as they do not perceive COVID-19 as a severe disease (Drążkowski & Trepanowski, 2021). Thus, we need evidence-based guidelines on attracting and motivating young adults in the most efficient and effective ways. Direct persuasion (providing arguments) usually is a less effective influence technique than self-persuasion (self-creation of arguments) (Aronson, 1999). As most young adults are not afraid to get COVID-19, self-persuasion targeted at others' health may be an effective method of increasing vaccination rates in this group. Thus among young adults, empathy seems to be a crucial factor affecting vaccination intention (Pfattheicher et al., 2021). In two experiments, using the Theory of Planned Behavior (TPB; Ajzen, 1991), we examined whether self-persuasion targeted at others' health can successfully encourage young adults to vaccinate against COVID-19. Moreover, we examined whether and how empathy is related through TPB components with the intention to vaccinate against COVID-19 (Pfattheicher et al., 2021).

METHODS
Study 1 (N = 366) compared the effectiveness of self-persuasion targeted at others' health to self-persuasion targeted at personal health and direct persuasion in encouraging vaccination intention. Study 2 (N = 375) investigated the applicability of self-persuasion in health communication through a poster framed as an open-ended question. In both studies, after the experimental manipulation, we measured the TPB component (utility, control, social, and moral norms beliefs; behavioral intention) in relation to COVID-19 vaccination. Both studies were conducted online among Polish Internet users aged 18-30. We analyzed the collected data using structural equation modeling.

RESULTS
In Study 1, we found that among young adults, self-persuasion targeted at others' health (compared to other forms of persuasion) have an indirect effect on vaccination intention against COVID-19 through utility and social norms beliefs. We found that as empathy increases, utility, social norms, and control beliefs increase, which in turn is associated with greater intention to vaccinate against COVID-19. Study 2 demonstrated that the poster with self-persuasion targeted at others' health enhanced vaccination intention, compared with a direct persuasion poster.

CONCLUSIONS
Together, our findings demonstrated the potential of self-persuasion targeted at others' health for health campaigns to increase COVID-19 vaccination uptake for young adults. Thus, our study contributes to the list of evidence-based methods of encouraging people to vaccinate against COVID-19. Further, our findings provide insight into why people are motivated to vaccinate against COVID-19 and accordingly contribute to the scientific pursuit of encouraging young adults to get vaccinations. Finally, our research has not only theoretical but also practical consequences, as we demonstrated that media posters with messages framed as open-ended questions provoking self-persuasion targeted at others' health could influence greater intention to vaccinate against COVID-19.
The systemic inflammatory response to influenza A virus infection is fueled by endothelial cells

**Topic:** Viral and host factors in the pathogenesis of influenza, RSV disease and COVID-19

Lisa Bauer, Laurine Rijssbergen, Lonneke Leijten, Feline Benavides, Rory de Vries, Rik de Swart, Debby van Riel

Erasmus Medical Center, Netherlands

**INTRODUCTION**

Influenza A virus infections cause in general mild and self-limiting upper respiratory tract disease in the majority of cases, but severe lower respiratory tract disease is the primary complication of influenza. Severe infections are often associated with dysregulation of systemic inflammatory responses in the lungs and circulation. Even though the primary target cells of influenza A viruses in humans are respiratory epithelial cells, it is becoming increasingly clear that endothelial cells play a key role in the systemic pathogenesis, especially in modulating systemic inflammatory responses. The exact mechanism underlying the contribution of endothelial cells to these responses is barely understood. Here, we investigate the contribution of endothelial cells to the systemic inflammatory responses after inoculation with seasonal or pandemic influenza A viruses.

**METHODS**

To mimic the pulmonary endothelium and to study the contribution of endothelial cells to the systemic inflammatory response in a relevant in vitro model, we made use of a transwell system in which primary lung epithelial cells on apical side are co-cultured with primary lung microvascular endothelial cells (LMEC) on the basolateral side. The well-differentiated organoid derived (distal airways) lung epithelial cells were cultured at air-liquid interface. In epithelial-endothelial co-cultures, we compared the susceptibility to infection and the associated immune responses of the pandemic virus H1N1 2009 (pH1N1) and the two seasonal virus strains H1N1 and H3N2 isolated in 2019.

**RESULTS**

Despite the fact that influenza A virus nucleoprotein could be detected in LMECs inoculated with influenza A virus, there was no evidence for the production of infectious progeny virus from endothelial cells. In epithelial-endothelial co-cultures, where epithelial cells were inoculated with influenza A virus, this did not result in infection of LMECs (based on expression of nucleoprotein) despite abundant infection of epithelial cells and breakdown of the epithelial barrier. In LMEC single cultures inoculation of virus triggered a modest cytokine response (IL-6, IL-8, IP-10). However, when we infected co-cultures with epithelial cells, a stronger immune response was observed in the LMECs compared to single LMEC cultures. This response consisted of type-I IFN (IFN-a, IFN-b), IP-10 and several interleukins (IL-1b, IL-6, IL-8, IL-10). In general, pH1N1 infected more cells and responses were stronger after inoculation compared to H1N1 virus and H3N2 virus isolated in 2019.

**CONCLUSION**

We show that endothelial cells are protected against infection when co-cultured with epithelial cells. However, the interaction with infected epithelial cells does amplify the inflammatory response in endothelial cells. In vivo, cytokines and chemokines produced by endothelial cells will directly enter the circulation, thereby contributing to the systemic cytokine response. Ongoing studies aim to identify factors that trigger inflammatory responses in endothelial cells.
Pediatric nasal epithelial cells are less permissive to SARS-CoV-2 replication compared to adult cells

**Topic:** Viral and host factors in the pathogenesis of influenza, RSV disease and COVID-19

Yanshan Zhu (1), Keng Yih Chew (1), Anjana Karawita (1), Ayako Yamamoto (2), Larisa Labzin (3), Tejasri Yarlagadda (4), Alexander Khromykh (1,5), Claudia Stocks (3), Yao Xia (6), Tobias Kollmann (7), David Martino (7), Anthony Kicic (7,8,9), Merja Joensuu (10,11), Frédéric Meunier (10,11), Giuseppe Balistreri (11,12), Helle Bielefeldt-Ohmann (1,13), Asha Bowen (7,14), Peter Sij (2,5), Kirsten Soann (4), Kirsty Short (1,5)

1: School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Australia;
2: Child Health Research Centre, The University of Queensland, South Brisbane, Australia;
3: Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia;
4: Centre for Immunology and Infection Control, Faculty of Health, School of Biomedical Sciences, Queensland University of Technology, Brisbane, Australia;
5: Australian Infectious Diseases Research Centre, Global Virus Network Centre of Excellence, Brisbane, Australia;
6: School of Science, Edith Cowan University, School of Biomedical Science, The University of Western Australia, Perth, Australia;
7: Wal-yan Respiratory Research Centre, Telethon Kids Institute, The University of Western Australia, Perth, Australia;
8: Occupation and Environment, School of Public Health, Curtin University, Perth, Australia;
9: Centre for Cell Therapy and Regenerative Medicine, School of Medicine and Pharmacology, The University of Western Australia, Perth, Australia;
10: Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane, Australia;
11: Queensland Brain Institute, The University of Queensland, Brisbane, Qld, Australia;
12: Department of Virology, Faculty of Medicine, University of Helsinki, Finland;
13: School of Veterinary Science, The University of Queensland, Gatton, Australia;
14: Department of Infectious Diseases, Perth Children’s Hospital, Nedlands, Perth, Western Australia

**BACKGROUND**

Children typically experience more mild symptoms of COVID-19 when compared to adults. There is a strong body of evidence that children are also less susceptible to SARS-CoV-2 infection with the original Wuhan isolate. The reasons for reduced SARS-CoV-2 symptoms and infection in children remain unclear and may be influenced by a multitude of factors, including differences in target cell susceptibility and innate immune responses.

**METHODS**

We used primary nasal epithelial cells from children and adults, differentiated at an air-liquid interface, to investigate differential infection kinetics and antiviral responses to SARS-CoV-2 infection. Viral replication was quantified by plaque assay. ACE2 protein expression were quantified by Western Blot and immunofluorescence. The cellular transcriptome of infected and uninfected cells was assessed by RNA-sequencing.

**RESULTS**

Here, we use primary nasal epithelial cells from children and adults, differentiated at an air-liquid interface, to show that SARS-CoV-2 (both the Wuhan isolate and the more recent Alpha variant) replicates to significantly lower titers in the nasal epithelial cells of children compared to those of adults. This was associated with a heightened antiviral response to SARS-CoV-2 in the nasal epithelial cells of children. Importantly, influenza virus, a virus whose transmission is frequently associated with pediatric infections, replicated in both adult and pediatric nasal epithelial cells to comparable titres.

**CONCLUSION**

We report significantly higher SARS-CoV-2 replication in adult compared to pediatric nasal epithelial cells. Pediatric nasal epithelial cells mount a strong anti-viral response to SARS-CoV-2 compared to adult cells. Taken together, our data suggest that the nasal epithelium of children supports lower infection and replication of SARS-CoV-2 than the adult nasal epithelium.
Patterns of liver cytolysis and lipid abnormalities in patients with mild and moderate COVID-19

**Topic:** Viral and host factors in the pathogenesis of influenza, RSV disease and COVID-19

Oana Sandulescu (1,2), Anca Streinu-Cercel (1,2), Victor Daniel Miron (1), Catalin Gabriel Apostolescu (1,2), Maria Nitescu (1,2), Anca Cristina Draganescu (2), Adrian Streinu-Cercel (1,2)

1: Carol Davila University of Medicine and Pharmacy, Bucharest, Romania; 2: National Institute for Infectious Diseases "Prof. Dr. Matei Bals" Bucharest, Romania

**BACKGROUND**

COVID-19 is increasingly recognized as a multisystem organ disease. Liver and lipid metabolism alterations have been described in severe and critical COVID-19, but their dynamics are less characterized for patients with mild-to-moderate disease.

**METHODS**

We performed a retrospective study of consecutive adult outpatients with mild and moderate COVID-19 evaluated in our clinic during the second and third waves of COVID-19 in Romania – November 2020 to April 2021. We assessed the dynamics of liver function tests and the occurrence of dyslipidemia at diagnosis and at days 7, 14 and 28.

**RESULTS**

The study included 524 patients with mean±SD age 51±13 years, and male-to-female ratio 1.1:1. At diagnosis, 29.2% of them presented high alanine aminotransferase (ALT), and almost half developed liver cytolysis over the course of evaluation, occurring significantly more frequently in males at all time points (Figure-1). ALT values were directly correlated with age ($p=0.001$, $r_s(518)=0.2$). High AST levels accompanied high ALT levels in only 58.5% of cases at diagnosis, and in 56.8%, 34.9% and 25.5% of cases at subsequent evaluations.

Overall, 30.4% of patients presented hypercholesterolemia at diagnosis, and the rate increased up to 56.0% by day 28 (Figure-1). Decreased HDL cholesterol levels were seen in up to half of patients and the values were inversely correlated with age ($p=0.013$, $r_s(489)=-0.1$), while hypertriglyceridemia was reported in one third of patients and the values were directly correlated with age ($p=0.021$, $r_s(486)=0.2$). With the exception of total cholesterol, all evaluated lipid abnormalities were significantly more frequent in males compared to females ($all\ p<0.05$ – Figure-1).

Interestingly, high baseline ALT and AST values appeared to be protective for developing lipid abnormalities by day 28. Specifically, they were protective against low HDL cholesterol levels ($p=0.001$, OR =2.5, 95%CI:1.6-3.8 for ALT and $p=0.001$, OR =2.6, 95%CI:1.7-4.1 for AST) and hypertriglyceridemia ($p<0.001$, OR =3.4, 95%CI:2.2-5.2 for ALT and $p=0.001$, OR =2.7, 95%CI:1.7-4.2 for AST), but not against hypercholesterolemia ($p=0.763$ for ALT and $p=0.287$ for AST).

**CONCLUSIONS**

In a cohort of patients with mild-and-moderate COVID-19, we have shown that liver cytolysis and lipid abnormalities can occur in up to half of the cases, particularly in males and directly correlated with advanced age. Furthermore, liver cytolysis at baseline appears to be protective against development of lipid abnormalities by day 28. This data could be used to inform clinical decision making and to better direct therapeutical interventions in patients with COVID-19 at risk of developing lipid abnormalities.

**Acknowledgement** All authors contributed equally.

*Figure 1. Patterns of liver enzyme and lipid metabolism abnormalities in patients with mild/moderate COVID-19*
RSV activating protease activity is elevated in bronchopulmonary dysplasia (BPD) affected lungs

**Topic:** Viral and host factors in the pathogenesis of influenza, RSV disease and COVID-19

Miriam Ruth Heindl (1), Judith Behnke (2), Lisa Rekers (3), Misa Gunjak (3), Torsten Steinmetzer (4), Rory Morty (3), Harald Erhardt (2), Eva Böttcher-Friebertshäuser (1)

1. Institute for Virology, Philipps University Marburg, Germany;
2. Department of General Pediatrics & Neonatology, Center for Pediatrics and Youth Medicine, Justus Liebig University, Gießen;
3. Department of Lung Development and Remodeling, Max Planck Institute for Heart and Lung Research, Bad Nauheim;
4. Institute for Pharmaceutical Chemistry, Philipps University Marburg

Bronchopulmonary Dysplasia (BPD) is a common complication of premature birth. Histopathology is characterized by a stunted lung development. The lungs of BPD affected children consist of larger alveoli in lower numbers and thicker septal walls compared to healthy children’s lungs. The overall respiratory epithelium is simplified and the high concentrations of O₂; mechanical ventilation and infections lead to inflammation of the tissues. BPD increases the susceptibility to viral infections of the respiratory tract even in adulthood and is known as a risk factor for severe courses of viral respiratory tract infections including infections with respiratory syncytial virus (RSV). The fusion protein F of RSV mediates membrane fusion between virus and host upon viral entry. The protein is synthesized as an inactive precursor and requires proteolytic cleavage by a host cell protease to gain fusion competence. Proteolytic cleavage is essential for viral infectivity. The RSV F-Protein is cleaved by the proprotein convertase furin. Here, we investigated whether BPD impacts the expression of potential RSV F activating proteases in the airways and thereby supports enhanced susceptibility to infection and virus activation and replication in BPD. We found that the activity of furin-like proteases was elevated in the lungs of a murine BPD model as well as in nasopharyngeal secretions of premature born children rehospitalized for viral infections. The nasopharyngeal secretions were able to support cleavage of RSV F in vitro. These findings suggest that BPD represents an environment benefiting RSV F activation. Moreover, furin-like activity in nasopharyngeal secretions of rehospitalized preterm children was efficiently reduced by peptide mimetic inhibitors of furin. Together, our data show that furin-like activity is increased in BPD affected lungs and might contribute to enhanced activation and pathogenicity of RSV infection.


*Institute for Virology, Philipps University Marburg +Department of General Pediatrics & Neonatology, Center for Pediatrics and Youth Medicine, Justus Liebig University, Gießen #Department of Lung Development and Remodeling, Max Planck Institute for Heart and Lung Research, Bad Nauheim *Institute for Pharmaceutical Chemistry, Philipps University Marburg
SARS-CoV-2 S1/S2 cleavage site mutants showed increased infectivity and fusogenicity suggesting enhanced TMPRSS2 mediated virus entry

**Topic:** Viral and host factors in the pathogenesis of influenza, RSV disease and COVID-19

Ritika Khatri
THSTI India

**BACKGROUND**
The spike (S) protein of SARS-CoV-2 virus binds to the host cell receptors which facilitates the virus entry. This interaction is primed by host cell proteases like furin and TMPRSS2 acting at S1/S2 and S2’ cleavage sites, respectively. Both the cleavage sites have Serine and Proline residues conserved in all the coronaviruses. Noticeably, highly transmissible SARS-CoV-2 variants, B.1.1.7 and B.1.617 also have mutation of P681H and P681R at S1/S2 site, respectively. It has been speculated that mutations at these conserved residues may provide a gain-of-function, easing the SARS-CoV-2 entry into the host cell and cell-to-cell spread, thus modulating the virulence and pathogenicity. Unravelling the effects of these conserved residues in the S protein cleavage site in virus entry and transmission might facilitate development of novel therapeutics.

**METHODS**
This study employed a lentivirus based pseudovirus (PSV) system, where P and S residues at S1/S2 site of Spike gene, present in an expression vector, were mutated to Alanine (Fig A). P681 site was also mutated to Histidine to study the effect of this amino acid mutation as present in B.1.1.7 variant. We then assessed the expression of the SARS-CoV-2-S and variants in HEK293T cells and tested the infectivity and fusogenicity of mutant PSVs in the presence or absence of S1/S2 and S2’ protease inhibitors.

**RESULTS**
SARS-CoV-2 variants were found to be robustly expressed and there was enhanced cleavage of spike proteins as compared to the wild-type SARS-CoV-2 spike protein (Fig B). As compared to hACE2, in presence of TMPRSS2, the mutants’ spike proteins were found to be highly fusogenic as compared to the wild type spike protein (Fig C). Wild type spike does not require presence of protease to fuse as compared to the mutant variants. P681H mutant expressed robustly than all other mutants. It showed the highest infectivity and fusogenic capacity. In presence of protease inhibitors, the P681H mutant showed significant reduction in infectivity and fusion inhibition (E-690 about 40% and Camostat Mesylate about 80% inhibition) respectively (Fig C and D).

**CONCLUSIONS**
Presence of proteolytic cleavage site is a key determinant of Enveloped viruses’ infectivity and pathogenesis. Our study suggests mutations in S1/S2 cleavage site and natural occurring P681H mutation favours viral entry by TMPRSS2 receptor most probably by augmenting spike protein cleavage by endosomal cysteine proteases Cathepsin B and Cathepsin L.
Abortive infection of neurons by seasonal and pandemic influenza viruses

Topic: Viral and host factors in the pathogenesis of influenza, RSV disease and COVID-19

Feline Benavides (1), Lisa Bauer (1), Bas Lendemeijer (2), Steven Kushner (2), Femke de Vrij (2), Debby van Riel (1)

1: Department of Viroscience, Erasmus MC, the Netherlands; 2: Department of Psychiatry, Erasmus MC, the Netherlands

BACKGROUND
Infection with Influenza A virus is generally mild and self-limiting. However, central nervous system (CNS) complications can occur in the acute phase of infection and occasionally in the post-acute phase. Evidence from humans and animals suggest that influenza viruses can enter the CNS through cranial nerves although the frequency varies among different subtypes. Seasonal and pandemic influenza viruses have occasionally been detected in experimentally inoculated animal and human cases suggesting that these viruses can enter the CNS. Once inside the CNS, the cell tropism, replication efficiency, and associated immune response of seasonal or pandemic influenza viruses is largely unknown. Thus, the aim of our study was to investigate the interaction of seasonal and pandemic influenza viruses with cells of the CNS using a human induced pluripotent stem cell (hiPSC)-derived neural model.

METHODS
We employed a rapid differentiation protocol to differentiate hiPSCs to neurons by forced overexpression of the transcription factor Neurogenin (Ngn2). Additionally, we directed hiPSCs through neural progenitor cells into astrocytes. Subsequently, a co-culture of neurons with astrocytes was made that support the survival and maturation of neurons. Co-cultures were infected with pandemic (2009 pH1N1) and seasonal influenza viruses (H1N1 and H3N2 isolated in 2019) after which we determined the cell tropism, replication kinetics and associated immune responses.

RESULTS
Inoculation of neural co-cultures with pH1N1, H1N1 2019 or H3N2 2019 viruses did not result in the production of progeny virus, detected by virus titration, despite the increase of viral RNA, detected by qPCR. Immune fluorescence staining of the neural co-cultures revealed infection of neurons after infection with all three viruses, although pH1N1 virus and H1N1 2019 virus infected more neurons than H3N2 virus.

As the hiPSC-derived neural co-cultures can be cultured for up to 10 days, we monitored virus infection over time. We detected virus-infected neurons up to 10 days post infection (dpi), without evidence for the production of infectious virus. The number of infected neurons remained stable in time, without morphological evidence for cell death. So far, we could not detect induction of anti-viral or proinflammatory cytokines during the course of disease. Ongoing experiments will elucidate the role of important cytokines for neuroinflammation and how replication is halted in neurons.

CONCLUSION
Seasonal and pandemic influenza viruses infected predominantly neurons up to 10 dpi. Despite evidence for RNA replication, infectious virus particles were not produced in the supernatant of the neural co-cultures. Altogether, our data so far suggest that neurons become infected and stay infected up to 10 dpi, without evidence for ongoing virus spread in the cultures or the induction of cell death. Further studies aim to characterise the associated immune responses in time and the long-term effect of the influenza virus infection on individual neurons.
Severe acute respiratory illness (SARI) in elderly patients admitted to an infectious diseases hospital in Romania during three pre-pandemic seasons, 2017/18, 2018/19 and 2019/20

**Topic:** Viral and host factors in the pathogenesis of influenza, RSV disease and COVID-19

Anca Cristina Draganescu (1), Victor Daniel Miron (2), Anca Streinu Cercel (1,2), Simona Paraschiv (1), Leonida Barica (1), Marius Surleac (1), Ovidiu Vlaicu (1), Andra Blascu (1), Bianca Enciu (2), Dan Otelea (1), Daniela Pitigoi (1,2), Adrian Streinu Cercel (1,2), Oana Sandulescu (1,2)

1: National Institute for Infectious Diseases "Prof. Dr. Matei Bals", Romania;
2: Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

**BACKGROUND**

Severe acute respiratory illness (SARI) represents one of the main drivers of infectious diseases-related morbidity during the cold season and elderly patients represent a patient population at particularly high risk of complications due to SARI. Each year during the influenza surveillance season (November to April), we perform screening for influenza and RSV in patients hospitalized for SARI in our tertiary case hospital for infectious diseases.

**METHODS**

Based on the data gathered through active epidemiological surveillance during three consecutive pre-pandemic influenza seasons (2017/18, 2018/19 and 2019/20), we have performed a case-control study of elderly compared to non-elderly patients admitted to the hospital for SARI. Comparisons between categorical variables were performed with the z tests for proportions.

**RESULTS**

In the three seasons, elderly patients (n=417) represented 8.8%, 14.5% and 9.9% of the total number of patients (n=3556) admitted to the hospital for SARI. Elderly patients presented higher rates of clinical findings suggestive for severity at hospital admission. Specifically, hypoxia was present in 10.3% of elderly patients compared to 6.2% in non-elderly patients (p=0.003, z=3.0), and confusion in 7.9% vs. 2.3% (p<0.001, z=6.4). Over the course of hospitalization, mechanical ventilation was needed in 1.9% vs. 0.3% of patients (p<0.001, z=4.3) and death was recorded in 3.1% vs. 0.3% of cases (p<0.001, z=7.2).

The overall rate of testing positive for influenza was 40.0% vs. 47.3% (p=0.020, z=2.3) in elderly vs. non-elderly patients, and specifically for influenza A 33.7% vs. 30.6% (p=0.05), and for influenza B 6.3% vs. 16.7% (p<0.001, z=4.6). For RSV the PCR positivity rate was 2.9% vs. 7.2% (p=0.001, z=3.3).

When specifically analyzing the group of elderly patients, we found that patients who tested positive for influenza had higher rates of hypoxia on hospital admission (21.1% vs. 11.1%, p=0.021, z=2.3) compared to patients with SARI of a different etiology.

**CONCLUSIONS**

Elderly patients with SARI may present warning signs of severity such as confusion or hypoxia at hospital admission more frequently compared to younger patients, requiring prompt diagnosis, timely initiation of treatment and close clinical monitoring during hospitalization.

**Acknowledgments**

All authors contributed equally.

**Funding**

GIHSN (Foundation for Influenza Epidemiology), DRIVE (EU/EFPIA IMI Joint Undertaking), NID.
Lipid peroxidation implication in COVID-19 severity.

**Topic:** Viral and host factors in the pathogenesis of influenza, RSV disease and COVID-19

**Authors:** Maria Martín-Fernández (1,2), Rocío Aller (1,2,3), María Heredia-Rodríguez (1,4,5), Esther Gómez-Sánchez (1,4,6), Pecio Martínez-Paz (1,6), Hugo González-Benítez (1,7), Laura Sanchez de Prada (8), Óscar Gorgojo (1,7), Irene Carnicero-Frutos (1,7), Eduardo Tamayo (1,4,6), Álvaro Tamayo-Velasco (1,9)

**Affiliations:**
1. BioCITIC Group for Biomedical Research in Critical Care Medicine, 47005 Valladolid, Spain;
2. Department of Medicine, Dermatology and Toxicology, Faculty of Medicine, Universidad de Valladolid, 47005 Valladolid, Spain;
3. Gastroenterology Department, Hospital Clínico Universitario de Valladolid, 47003 Valladolid, Spain;
4. Department of Surgery, Faculty of Medicine, Universidad de Valladolid, 47005 Valladolid, Spain;
5. Anesthesiology and Critical Care Department, Hospital Clínico Universitario de Salamanca, 37007 Salamanca, Spain;
6. Anesthesiology and Critical Care Department, Hospital Clínico Universitario de Valladolid, 47003 Valladolid, Spain;
7. Institute of Health Sciences of Castile and Leon (IECSCYL), 47002 Soria, Spain;
8. NIC VALLADOLID-HCUV Spain;
9. Haematology and Hemotherapy Department, Hospital Clínico Universitario de Valladolid, 47003 Valladolid, Spain.

**BACKGROUND**

Severe COVID-19 infection triggers imbalanced and uncontrolled cytokine response, exuberant endothelial inflammatory reactions and vascular thrombosis. Oxidative stress may be a key player in COVID-19 pathogenesis due to its significant role in response to infections. A defective redox balance has been related to viral pathogenesis developing a massive induction of cell death provoked by oxidative stress. The aim of this study is to evaluate lipid peroxidation role in severity of this disease.

**METHODS**

Blood samples were obtained from 108 COVID-19 patients and 28 controls and lipid peroxidation levels were assessed and compared between COVID-19 and Non-COVID-19 patients as well as based on severity of COVID-19 infection. Multivariable regression analysis was performed to identify the independent association between lipid peroxidation levels and intubation/death risk in COVID-19 infection. Probability of intubation/death to day-28 in COVID-19 patients based on LPO OOP was analyzed by using Kaplan-Meier curves and tested with the log-rank test.

**RESULTS**

Lipid peroxidation levels were significantly higher in COVID-19 patients, increasing with disease severity (Figure 1). COX multivariate regression analysis identified LPO levels over the OOP (LPO>1948.17 µM) as an independent risk factor for 28-day intubation/death in COVID-19 patients [OR: 2.57, 95% CI: 1.10–5.99; p=0.029] (Table 1). Furthermore, Kaplan-Meier curve analysis revealed that COVID-19 patients showing LPO levels above 1948.17 µM were intubated or died 8.4 days earlier on average (mean survival time 15.4 vs 23.8 days) when assessing 28-day intubation/death risk (p<0.001) (Figure 2).

**CONCLUSIONS**

These findings revealed that higher lipid peroxidation levels are independently associated to a higher risk of intubation or death at 28 days in COVID-19 patients.

![Figure 1. Box plots showing LPO levels in COVID-19 and Non-COVID-19 patients as well across disease severity groups. The line represents significant differences between groups. The triangle represents significant differences against the healthy control.](image-url)
Table 1. Multivariate logistic regression analysis to evaluate the independent association of LPO levels and risk of intubation or death at 28 days.

<table>
<thead>
<tr>
<th>Intubated/Death COVID-19 disease</th>
<th>OR</th>
<th>CI 95%</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPO &gt; 1346.17 μM</td>
<td>2.57</td>
<td>1.15 - 5.80</td>
<td>0.029</td>
</tr>
<tr>
<td>Obesity</td>
<td>1.19</td>
<td>0.46 - 2.97</td>
<td>0.702</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2.61</td>
<td>1.20 - 5.69</td>
<td>0.017</td>
</tr>
<tr>
<td>Chronic hepatic disease</td>
<td>1.74</td>
<td>1.37 - 2.21</td>
<td>0.006</td>
</tr>
<tr>
<td>Septic shock</td>
<td>2.41</td>
<td>1.15 - 5.02</td>
<td>0.022</td>
</tr>
<tr>
<td>Lymphocytes × 876 cells/μl</td>
<td>0.22</td>
<td>0.10 - 0.53</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Neutrophils &lt; 5000 cells/μl</td>
<td>2.30</td>
<td>1.04 - 5.28</td>
<td>0.041</td>
</tr>
</tbody>
</table>

Figure 2. Kaplan-Meier curve analysis showing LPO association with intubation/death risk.
The role of viral polymerase segments in the pathogenicity of H7N7 avian influenza viruses in poultry

**Topic:** Viral and host factors in the pathogenesis of influenza, RSV disease and COVID-19

Diana I. Palme (1), David Scheibner (1), Maryna Kuryshko (1), Thomas C. Mettenleiter (3), Angele Breithaupt (2), Elsayed M. Abdelwhab (1)

1 Institute of Molecular Virology and Cell Biology, Friedrich-Loeber-Institut Federal Research Institute for Animal Health, Südufer 10, 17493, Greifswald, Insel Riems, Germany
2 Department of Experimental Animal Facilities and Biorisk Management, Friedrich-Loeber-Institut Federal Research Institute for Animal Health, Südufer 10, 17493, Greifswald, Insel Riems, Germany
3 Friedrich-Loeber-Institut, Federal Research Institute for Animal Health, Südufer 10, 17493, Greifswald, Insel Riems, Germany

**BACKGROUND**
Avian influenza viruses (AIV) of the subtype H7 can evolve from a low pathogenic (LP) precursor to a high pathogenic (HP) form in chickens, causing severe systemic infections and high mortality rates in infected flocks. A polybasic cleavage site (pCS) in the hemagglutinin (HA) surface protein of AIV is one of the main virulence determinants in high pathogenic AIV (HPAIV), whereas less is known about the role of the viral polymerase segments (PB2, PB1, and PA) in the transition of LP to HP AIV. In 2003, HPAIV H7N7 was isolated in poultry in the Netherlands and further spread to poultry farms in Germany and Belgium resulting in the death and culling of about 30 million birds. The HPAIV H7N7 infected more than 1000 humans and resulted in the death of an immunocompromised veterinarian. Previous studies showed that the 2003 HPAIV H7N7 is genetically related to contemporary LPAIV H7N7 circulating in wild birds in Europe. Furthermore, they indicated that both pCS-HA and polymerase genes play a role in virus virulence in mammals, whereas little is known about the virulence determinants of this virus in poultry.

**METHODS**
Here, we used reverse genetics to construct recombinant LP H7N7 viruses carrying a pCS (designated LP_Poly) with or without the polymerase segments PB2, PB1, or PA from HP H7N7. The recombinant H7N7 viruses were characterized in vitro using growth kinetics and plaque tests. In addition, the polymerase activity was determined in avian and mammalian cells using a dual reporter luciferase assay. In vivo, chickens were infected with the different recombinant viruses to assess virulence, transmission, and virus replication in tissues and swab samples.

**RESULTS**
In vitro, PB2, PB1, and PA had slight effects on the viral replication and spread in cell culture, polymerase activity and HA expression levels. In vivo, the pCS increased the virulence of LP H7N7 in chickens although at lower level compared to the HPAIV H7N7. Interestingly, the introduction of the HP H7N7 polymerase segments reduced the virulence of the recombinant viruses in comparison to LP_Poly. However, viral transmission from infected to contact chickens could be observed for all viruses except for LP_Poly_PB2. The shedding of the recombinant viruses mainly occurred via the cloacal route.

**CONCLUSION**
The HP H7N7 polymerase segments showed only minor effects on LP H7N7 virus replication in cell culture. The polymerase segments of HPAIV H7N7 did not increase the virulence of LPAIV H7N7, however, PB2 played a role in chicken-to-chicken transmission. It remains to study the impact of other gene segments (i.e., NA, NS, NP, and M) on virus virulence and transmission in chickens.
Viral and host factors in the pathogenesis of influenza, RSV disease and COVID-19

Viral and host factors in the pathogenesis of influenza, RSV disease and COVID-19

David Scheibner, Angele Breithaupt, Christine Luttermann, Diana Palme, Maryna Kuryshko, Claudia Blaurock, Thomas C. Mettenleiter, Elsayed M. Abdelwhab

Friedrich-Loeber-Institut, Germany

**BACKGROUND**

Avian influenza viruses (AIV) are a common threat to poultry and human health and cause periodically huge socioeconomic losses worldwide. Depending on the two major surface glycoproteins, 16 hemagglutinin (HA) and 9 neuraminidase (NA) distinct subtypes can be distinguished. All HA/NA subtypes are circulating in wild waterfowl, the natural reservoirs. In domestic birds, AIV are either low pathogenic (LPAIV) causing mild or no clinical signs or highly pathogenic (HP) causing up to 100% mortality in few days. In chickens, LPAIV H3 and H7 viruses can evolve to HPAIV due to acquisition of polybasic amino acids in the HA cleavage site (HACS). Conversely, ducks rarely succumb to morbidity and mortality after HPAIV infections and the HACS alone is not the main virulence determinant. Since 2014, H5N8 clade 2.3.4.4 caused high mortality in domestic and wild birds worldwide in three major waves in 2014, 2016 and 2020/2021. Although all H5N8 viruses possessed polybasic HACS and were highly virulent in chickens, viruses in 2016-2021 only were high pathogenic in Pekin ducks. In this study, the virulence determinants leading to differences in virus phenotypes between German H5N8 clade 2.3.4.4 viruses in 2014 and 2016 were analyzed.

**METHODS**

Sequence analysis of H5N8 viruses from 2014 and 2016 revealed several mutations in all eight gene segments. Using reverse genetics, single, double and multiple H5N8-2016 segments were swapped with those from H5N8-2014 and recombinant viruses were rescued and characterized. The minimal genetic constellation for the exhibition of high virulence in Pekin ducks was determined.

**RESULTS**

In vivo, the H5N8-2016 HA alone did not increase the virulence of H5N8-2014 in Pekin ducks. We further tested double reassortants of H5N8-2014 carrying HA in addition to single segments from H5N8-2016. Virulence of H5N8-2014 increased dramatically after reassortment of H5N8-2016 HA in combination with NP, NS and/or NA. The high mortality caused by these viruses was associated with the degree of endothelial tropism and systemic spread in different tissues. In vitro, the replication of the H5N8-2014 virus in primary duck cells was increased by swapping the HA and NP from H5N8-2016. The latter exhibited increased HA receptor binding affinity and NA activity compared to the H5N8-2014 virus.

**CONCLUSION**

Taken together, in this study we determined the multifactorial genetic constellation and underlying mechanism for the high virulence of H5N8 clade 2.3.4.4 viruses in Pekin ducks, which improves our current understanding for the evolution of HPAIV in different avian species.
**sa-mRNA quadrivalent seasonal influenza vaccines elicits robust neutralizing antibody and cell mediated immune response against all four strains**

**Topic:** Virus structure and replication in influenza virus, RSV and SARS-CoV-2; latest developments in influenza virus, SARS-CoV-2 and RSV molecular virology

Giuseppe Palladino, Gillis Otten, Ethan Settembre, Yingxia Wen

Seepius, United States of America

**BACKGROUND**

Seasonal quadrivalent influenza vaccine is designed to protect against four different flu viruses, including A/H1N1, A/H3N2, B/Victoria and B/Yamagata viruses circulating each flu season. The key antigen in the vaccines are Hemagglutinins (HAs) from four strains which elicit anti-HA neutralizing antibody response preventing viral infection of these strains. Neuraminidase (NA) is another influenza surface protein and a potential additional antigen which is not included or controlled in current flu vaccines.

**METHODS**

Alphavirus-based self-amplifying mRNA (sa-mRNA) encapsulated within a lipid nanoparticle (LNP) has been developed as a novel second-generation RNA vaccine platform technology. We developed a quadrivalent sa-mRNA vaccine co-expressing HA and NA antigens from four viruses.

**RESULTS**

Mouse immunogenicity studies demonstrated that the quadrivalent sa-mRNA HA-NA vaccines raised robust anti-HA and anti-NA neutralizing antibody responses against all four viruses. Sa-mRNA HA-NA vaccines also elicited a Th1-dominant antigen specific CD4+ T cell responses and robust and broad CD8+ T cell response to both HAs and NAs.

**CONCLUSIONS**

sa-mRNA seasonal quadrivalent influenza vaccines raised robust neutralizing antibody response and cell mediated immune response, suggesting the new generation of vaccine may be more effective against influenza infection in humans than current influenza vaccine.
Filamentous and spherical influenza virions have distinct structural and functional properties

**Topic:** Virus structure and replication in influenza virus, RSV and SARS-CoV-2; latest developments in influenza virus, SARS-CoV-2 and RSV molecular virology

Jack Hirst, Sweetha Vijayakrishnan, David Bhella, Ed Hutchinson

MRC-University of Glasgow Centre for Virus Research, United Kingdom

**BACKGROUND**

Most laboratory-adapted strains of influenza A virus (IAV) produce predominantly spherical virions. In contrast, clinical IAV isolates produce pleiomorphic virions with a continuous range of sizes from 0.1 micron spheres to filaments that can reach tens of microns in length. Influenza filaments have been known of since the 1940s, but they are complex, hard to handle, and rarely produced by laboratory strains. The basic properties of these striking features of natural IAV infections are therefore poorly understood. In this study we combined mass spectrometry (MS) and cryogenic electron tomography (cryo-ET) to describe the fundamental properties of IAV filaments.

**METHODS**

Virions of the filamentous IAV strain A/Udorn/307/72 (H3N2) (Udorn) were grown on MDCK cells. Confocal microscopy and automated image analysis were used to optimise a density gradient that separated virions based on morphology. The properties of virions with different morphologies were then determined by standard biochemical and virological methods and by MS proteomics. These bulk population data were combined with cryo-ET studies of individual virions.

**RESULTS**

Filamentous virions can be reproducibly separated from spherical virions due to slight differences in their density. When compared to spherical virions, filamentous virions were (by total mass) depleted in several viral proteins, which correlates with their functional properties. Filaments incorporate proportionally less NA, affecting the balance of their receptor binding and cleaving. They appear to incorporate less NS1 and viral genome-associate proteins, and have a reduced ability to initiate productive infections. While they incorporate similar levels of host proteins and of the matrix protein M1, their elongated dimensions can allow these components to form extended structural features that have not previously been described in influenza virions - coaxial layers of matrix and an internal cytoskeleton.

**CONCLUSIONS**

Influenza virions are complex and heterogeneous structures and cannot be studied in detail using any current method in isolation. By combining classical virology methods with advanced proteomic and structural biology approaches we have produced a detailed description of filamentous IAV virions, identifying multiple ways in which their strikingly elongated form drives their function.