EPIDEMIOLOGY WORKING GROUP

The epidemiology working group continues to work on generating comprehensive seroepidemiology templates for influenza. However, the continued occurrence of MERS-CoV cases in Saudi Arabia and the unprecedented Ebola outbreak in West Africa has shifted many of our attentions to these pressing and serious events.

As such, much of the focus of the epidemiology working group has been on another important respiratory infection: MERS-CoV. There are now three MERS-CoV seroepidemiology protocol templates on the WHO website. The three sero-epidemiology templates are based on protocols developed by CONSISE and can be found at the links below:

- Seroepidemiological Investigation of Contacts of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Patients
- Assessment of potential risk factors of infection of Middle East respiratory syndrome coronavirus (MERS-CoV) among health care personnel in a health care setting
- Cross-sectional seroprevalence study of Middle East respiratory syndrome coronavirus (MERS-CoV) infection in presumed high risk populations

These templates have been adapted for use in Qatar and Saudi Arabia and we hope to learn of the results of these studies soon. The MERS-CoV at risk population protocol will be used in Algeria and Morocco in the coming year. CONSISE members contributed to a fourth protocol - a case control study - which was initially proposed as a seroepodemiology study, but had the seroepidemiology component removed after consultation with MERS-CoV affected member states.

ROSES statement

CONSISE has produced the statement on Reporting of Seroepidemiologic Studies for Influenza (ROSES-I). The ROSES-I statement is an extension of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement, adding specific methodological details that should be reported in publications of influenza seroepidemiologic studies. The ROSES-I statement covers the reporting of details of the study methodology and laboratory methods including, for example, study design, study population, epidemiologic data collection, specimen collection and handling methods, laboratory methods, justification of criteria for seropositivity, reporting of results, limitations and biases, and interpretation in order to allow interpretation and comparison. The ROSES-I statement will be submitted to a peer-reviewed journal in the coming weeks.

SAVE THE DATE: Upcoming CONSISE Meeting for Epidemiology working group members

The International Society for Influenza and Other Respiratory Virus Diseases (ISIRV) is planning a 2-day meeting on incidence, severity and impact of influenza in Paris, 21-22 January 2016, and CONSISE will hold a 1-day meeting on 20th January to coincide with that event. The meeting will be hosted by Institut Pasteur. The ISIRV meeting will be a follow-up to a very successful meeting on a similar topic held in Munich in September 2012.

At the CONSISE meeting, we will present the online draft version of the question bank for CONSISE influenza protocol templates and the remaining influenza protocol templates undergoing revision

and finalization. A full agenda is currently being developed. If you are interested in participating, please contact Maria Van Kerkhove at maria.van-kerkhove@pasteur.fr.

LABORATORY WORKING GROUP

As reported in the second CONSISE newsletter, the main focus of the laboratory working group is to coordinate and standardize the international serology laboratory response to new emerging influenza viruses. Since April 2014, there has been significant progress in two CONSISE laboratory studies:

An international laboratory comparison of influenza microneutralisation assays for A(H1N1)pdm09, A(H3N2) and A(H5N1) influenza viruses

As reported in earlier CONSISE newsletters, we have compared two consensus protocols for MN; comparison of the 2-day ELISA-based and the 3-day haemagglutination (HA) assay-based methods in 10 laboratories using the A(H1N1)pdm09 strain demonstrated that there is a good correlation between the two methods in most laboratories and therefore either of these CONSISE consensus protocols could be recommended. These results were subsequently confirmed for both H3N2 and H5N1 viruses in an extension of the comparative study (H3N2 results from seven laboratories and H5N1 results from three laboratories). A report of the study was circulated to study participants for comment and a manuscript has now been submitted for publication.

A collaborative study to assess the variability of ELLA in measuring influenza virus neuraminidase inhibiting antibody titres

As reported in the second CONSISE newsletter, we have agreed to evaluate the reproducibility of the enzyme-linked lectin assay (ELLA) for detection of neuraminidase (NA) antibodies. CONSISE laboratory members were invited to participate in the study in April 2014. This included 15 laboratories with prior experience with ELLA and 20 laboratories without prior experience with this assay. These latter laboratories were provided with guidance to establish the assay prior to running study samples. Inactivated H6N1 and H6N2 antigens were prepared and shipped to participants, together with a standard assay and study instructions. Data were submitted for statistical analysis in 2 phases: Phase I: preliminary analysis of data submitted by experienced labs; Phase II: final analysis of data from all laboratories.
On Friday 19th September 2014, we held a "CONSISE workshop on influenza neuraminidase (NA) and discussion of an international study of the 'ELLA' NA inhibition assay" in Bethesda, USA which was an opportunity to evaluate and discuss the results from the Phase I analysis and to hear from experts in the field of NA immunity. Participants in the study were invited to the meeting but the meeting was also organised as a webinar. Presentations from the meeting are available to CONSISE members on the CONSISE website
https://globalhealthtrials.tghn.org/site_media/media/medialibrary/2014/10/CONSISE_NA_Meeting_tabular.pdf

Following completion of the Phase II analysis, a study report was compiled which has been circulated to all study participants for comment. A summary of the main findings is as follows:

- The ELLA assay was readily implemented by laboratories new to the assay
- There was good reproducibility of ELLA assays within laboratories
- NA titres reported by different laboratories varied substantially (8-fold or more differences; Geometric Coefficient of Variation 112% for N1 and 82% for N2 antigens)

- The variability in titre between laboratories was substantially reduced by inclusion of an appropriate antibody standard

It is noteworthy that the degree of variability between laboratories for the ELLA assay was less than that seen for other influenza serology assays (Haemagglutination-inhibition [HI] and MN) *(Stephenson et al, 2009; Wood et al 2012)*. This study has taken a significant step in establishing international harmonization to reduce inter-laboratory variability for the ELLA assay.

Future studies

It had been planned to start a new comparative study using the HI and the MN consensus assay protocols in the last few months of 2014, but this was not possible due to CONSISE laboratories being occupied with the ELLA study. This study is now planned for 2015 and will examine laboratory-to-laboratory variability using A(H1N1)pdm09 virus(es) and possibly other viruses when a panel of sera is tested in each laboratory. At the same time, various sources of potential antibody standards will be evaluated as described in the first CONSISE newsletter. A small study group has been established to develop the detailed study protocol.

CONSISE MEMBER HIGHLIGHT

Olav Hungnes Ph.D., Senior Scientist and Director, WHO National Influenza Centre for Norway, Department of Virology, Norwegian Institute of Public Health, Oslo, Norway.

Can you tell us a little about yourself?

Having always been interested in laboratory work, biology and natural history, I graduated with a master's degree in molecular cell biology from the University of Oslo in the mid-1980's. At the same time, my supervisor Bjørn Grinde was taking up a post in AIDS virus molecular research in the Department of Virology, Norwegian National institute of Public Health, an institute that I hardly knew existed at the time. Thus an opportunity arose for me to come with him as a Ph.D. student. The next few years I spent establishing a molecular virology lab unit for HIV, and studying some very minor details of HIV-1 reverse transcription. Work gradually moved more towards molecular epidemiology, and through the 1990s I also became involved in establishing the molecular diagnostics and sequence analysis side of the influenza surveillance. This period also saw my first involvement with influenza seroepidemiology, as I was tasked with managing the huge Excel spreadsheets with data from the annual influenza serosurvey. This survey has been performed every year since being spearheaded by Lars Haaheim in the late 1970s, but is carried out in our laboratory since the mid-1980s. In many years it was performed as a collaboration between the National Influenza Centre (NIC) for South-eastern, Mid- and Northern Norway led by Dr. Liv B. Flugsrud and the NIC for Western Norway led by Prof. Lars R. Haaheim in the University of Bergen. Upon the retirement of Dr. Flugsrud in 1999, I was asked take over as NIC director.

Assuming this function, I also proudly inherited the annual serosurvey. For a long period, Norway was one of a very few countries where influenza serosurveys were carried out on a regular basis. Yearly these analyses were applied at national level, used retrospectively as a complement to the virological surveillance carried out through the season, and prospectively in attempts to chart population or sub-population vulnerability to the different contemporary viruses. Outcomes of our serosurveys are also presented each year to the February WHO Influenza Vaccine Composition meetings.

With the emergence of the pandemic of 2009/10, interest in influenza seroepidemiology rose significantly and all of a sudden we were old-fashioned-turned-fashionable. We were able to document very low pre-existing prevalence of antibody reactive to the pandemic virus, a dramatic rise in immunity during the autumn months, and then how the newly gained immunity was sustained well in some age groups but waned over the next months in others. Furthermore, we have assessed the epidemic potential in the Norwegian population of the US swine-origin H3N2v that has been involved in many zoonotic infections and whose HA is derived from human H3N2 viruses in the mid-1990s, finding that sizeable age segments have antibody reactive to the H3N2v virus.

Looking back at my beginnings, it is clear that I have ended up on a branch of biology that I would never had foreseen, but nevertheless is the perfect place to witness natural history unfolding at a pace not seen anywhere else.

What is your involvement in CONSISE?

The need for better comparability between influenza seroepidemiology studies has been recognised for a long time and we were quite ready to embrace the CONSISE initiative when it came. Our most urgent need was for better laboratory method standardisation and comparability, but we are also in a position to contribute with our experience to the CONSISE protocol for seroepidemiology of Influenza and other viral respiratory infections using convenience residual sera. This dual role has found me shuttling back and forth between the lab and epi groups during some of our meetings. I am also a member of the CONSISE Steering Committee, where I find myself in the company of very eminent world class experts, and I suppose my unique contribution in this context is to represent the perspective of those who need to make influenza seroepidemiology work in more basic settings.

How do you see CONSISE adding to the standardization of serologic assays for influenza?

In seroepidemiology, we are attempting to use immunological parameters to find out things like who has been exposed, and who are likely to be protected, against a given pathogen. We are thus trying to get simple answers out of a very intricate biological interplay between antigens that in themselves may be poorly defined, and a hugely complex immune response in individuals that may have seen many antigenic variants throughout life. The immune parameters we measure are also a just a part of the concerted immune response and the different parts may vary in importance among individuals. Because of this it is a formidable task to make the serological analyses give relevant and dependable answers to these basic questions. We therefore need to manage expectations on what can be expected, but at the same time there is a lot that we can achieve to enhance the utility of our assays.

We have already made very good progress with the establishment of consensus protocols, making use of international standards, in comparative work on the neutralisation assays, and with the neuraminidase ELLA assay work now being finalised.

Looking forward, on the short term I am looking very much forward to the upcoming comparative exercise for the HI assay, since this remains the single most widely utilised seroepidemiology assay for influenza, and perhaps also the least standardised. I think many CONSISE members are waiting for this exercise which I think targets a need at the very core of our mission.

Further onwards, I think more work is needed to give guidance on how to interpret the readouts of the assays in terms of the research questions asked, where to draw the line for exposure, and where for immunity. Can we even have one cutoff across all age groups?

Not losing sight of our mission, I think we also need to connect with those who are applying the corresponding assays for other purposes, such as response to different vaccines and correlates of protection.

References

Stephenson I, Heath A, Major D, Newman RW, Hoschler K, Junzi W, Katz JM, Weir JP, Zambon MC, Wood JM. Reproducibility of serologic assays for influenza virus A (H5N1). Emerg Infect Dis. 15(8):1252-9, 2009.

Wood JM, Major D, Heath A, Newman RW, Höschler K, Stephenson I, Clark T, Katz JM, Zambon MC. Reproducibility of serology assays for pandemic influenza H1N1: collaborative study to evaluate a candidate WHO International Standard. Vaccine 30(2):210-7, 2012.