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RESEARCH NOTE

Diagnosing Influenza for Research

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The impact of influenza on the global burden of illness and mortality has been recognised for centuries. In Dublin in 1837 Robert Graves attempted the first quantification of influenza-attributed mortality by counting new graves in the church cemetery following a severe influenza epidemic and comparing this number with the count from the previous year (Evans and Kaslow 1997: 474). Today a robust evidence base is essential to underpin clinical and public health policies to combat influenza.

Colds and flu are an almost universal human experience, yet identifying true influenza infections for research is surprisingly challenging. Whether a research project is an observational study of influenza risk factors or a randomised controlled trial of new preventive measures, imprecise case definitions can undermine results. Once someone becomes infected with influenza their place on the twin spectra of illness severity and health behaviour will influence the likelihood of diagnosis. Some people become infected and produce antibodies against influenza but have no symptoms. Others become ill but do not seek medical advice, preferring instead (often rightly) to self-treat with measures such as rest, rehydration and paracetamol. Neither group would be identified by studies drawing their samples from general practice or hospital records. Asymptomatic infections would be identified only in studies using seroconversion (or antibody production) as an outcome. For researchers to detect infections producing minor clinical symptoms people would need to participate in community studies using active surveillance in which they are regularly quizzed about symptoms. Examples include Flu Watch, a large national household cohort study of behavioural and biological determinants of influenza transmission, and Flu Survey, part of a Europe-wide initiative gathering online data about influenza trends in the UK (*Flusurvey*).

Once symptoms become more severe people may seek medical attention. In a clinical setting like general practice a diagnosis of influenza is typically considered if a patient presents with fever and sudden onset of respiratory symptoms during the influenza season, i.e. time periods when influenza virus is circulating (*Centers for Disease Control*). The same symptoms would yield a much lower positive predictive value (or proportion of 'true positives') when prevalence of circulating virus is low. An influenza diagnosis might also be considered in certain categories of person with atypical symptoms, e.g. the elderly or immuno-compromised. The majority of these patients will not be tested as testing will not alter clinical management. From a research perspective the code entered by a GP into a patient's record will be taken as the diagnosis. Codes based on clinical symptoms are attached to descriptive labels ranging from the fairly precise, e.g. 'influenza-like illness' – which even when true to the Centers for Disease Control and World Health Organization definition has modest sensitiv-

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ity (around 65%) and specificity (67%) for diagnosing influenza (Petrozzino and others 2010) – to the very general, e.g. 'chest infection'. People at high risk of complications, including the elderly and those with chronic medical conditions, may receive investigations and/or anti-viral treatment. These people are perhaps more likely to have an influenza-specific diagnostic label entered into their notes than those with less worrying symptoms – a potential source of bias in research studies.

With increasing symptom severity or the development of complications a hospital admission may result. Here diagnostic tests are performed more frequently, but these patients form the 'tip of the iceberg' of severity and are not representative of the majority with influenza; care should be taken when studying them. The highest point of the iceberg signifies patients who die from influenza or its complications. However, many deaths attributable to influenza arise from exacerbations of underlying conditions such as cardiovascular disease or complications such as bacterial pneumonia. The diagnosis 'influenza' may not be recorded on a patient's death certificate or even considered by treating physicians. When research studies quantify influenza-attributed deaths, complex mathematical models are needed to overcome the extreme underestimation that would result from only including cases where influenza is listed as the primary cause of death.

In primary research, rather than studies based on secondary data, surely (mis)diagnosis is less of an issue? This is true to some extent, although no existing test is perfect. Using clinical case definitions of influenza-like illness will miss a substantial proportion of people who are either asymptomatic or fail to meet diagnostic criteria, whilst falsely diagnosing some with influenza-like symptoms caused by other organisms such as respiratory syncytial virus or rhinovirus. Asking people to recall symptoms within a specified time frame, e.g. the last week or last month, can produce various inaccuracies. For example, in a case control study cases (or people with a specified disease) may remember recent respiratory symptoms differently to controls (or those without the disease being studied), which is an example of recall bias.

Laboratory influenza testing is potentially more reliable. The two main strategies are to isolate the virus or fragments of it directly from bodily secretions or to measure the body's immune response to infection. Types of respiratory specimens used for diagnosis range from nasal swabs to nasopharyngeal swabs and aspirates – the more invasive the test, the better the sensitivity, although potentially the less acceptable to research participants. The traditional gold standard for influenza diagnosis was viral culture (Kumar and Henrickson 2012). While the long turnaround times (3-10 days) make this less useful in clinical settings it is still a valid method for research. Nowadays viral culture has been somewhat superseded by reverse-transcriptase polymerase chain reaction (RT-PCR) – considered the most sensitive and specific test for influenza – which produces results in hours rather than days (Kumar and Henrickson 2012). However, having a positive RT-PCR test depends upon there being sufficient viral shedding, or expulsion of infectious virus particles from an infected person in respiratory secretions. People infected with influenza shed virus maximally within the first two days of infection and may have ceased to produce virus by five days (Carrat and others 2008). In research studies sufficient resources and procedures must be in place for rapid sample collection after symptom onset, otherwise the utility of this test will fall significantly.

Since the 2009 H1N1 influenza pandemic rapid influenza diagnostic tests have proliferated, driven by a need for prompt diagnosis in clinical settings. These are often based on immunoassays that use antibodies labelled with coloured tags to recognise influenza virus antigen in respiratory specimens (Chartrand and others 2012). However,

although specific results may be produced at the bedside within fifteen to thirty minutes, sensitivity is often poor (Chartrand and others 2012); accuracy may be the trade-off for rapidity. Finally, seroconversion, denoted by a four-fold rise in influenza antibody titre in two separate serum samples taken a few weeks apart, is rarely used in clinical practice because of lack of timeliness but may be useful for research. As well as the logistic and cost implications influenza vaccination will cause a rise in antibody titre, so an accurate vaccination history is essential to interpret results.

So what is the best way to diagnose influenza for research? The most appropriate tests will depend on the research question and study design as well as on budget. Usually a combination of measures is most robust. Sometimes creativity is needed, for example using national influenza surveillance data to ascertain time periods when influenza is circulating and when an influenza-like illness is therefore most likely to be due to flu.

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