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Satisfying the need for rapid diagnosis of new variant influenza A H1N1

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"Decisions concerning antiviral therapy and quarantine measures require rapid results, since antiviral therapy should preferably be initiated within 24 h after the appearance of the patient's first clinical symptoms."

Experience gathered during the first phase of the new variant influenza H1N1 (MexFlu) pandemic confirmed that the diagnostic need in the management of MexFlu-infected patients is required for the institution of antiviral therapy and quarantine measures of suspected cases, and for the evaluation of antiviral therapy. Decisions concerning antiviral therapy and quarantine measures require rapid results, since antiviral therapy should preferably be initiated within 24 h after the appearance of the patient's first clinical symptoms [1]. The diagnostic armamentarium contains so-called rapid tests detecting influenza antigens, virus culture, antibody detection and molecular techniques (e.g., reverse transcriptase [RT]-PCR). Rapid tests are, in general, not sensitive for new influenza strains and they do not distinguish between seasonal and new pandemic strains. Virus culture and serology do not provide results within 1 working day.

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Historically, RT-PCR based on readout agarose gel techniques with subsequent hybridization for specificity, is labor intensive and not suited for generating data on large numbers of samples on a daily basis. The introduction at the end of the last century of (semi)automated nucleic acid-extraction systems and real-time RT-PCR allowed diagnostic laboratories to perform inhouse-developed assays for detecting and typing viruses on large numbers of samples on a daily basis and generate results within 5–12 h after receipt of the sample in the laboratory. Real-time RT-PCR is, furthermore, capable of generating quantitative results, which can be used to evaluate antiviral therapy in an individual patient.

The appearance of circulating influenza virus strains resistant to antiviral drugs and the development of resistant viruses during antiviral therapy has become a major concern in influenza patient management. In recent years, influenza A virus subtypes H3N2 and the pandemic H1N1 are naturally resistant to the antiviral drugs (amantadine and rimantadine). In addition, since the epidemic of 2007–2008, seasonal influenza A/H1N1 virus has become resistant to the neuraminidase inhibitor oseltamivir. In patients with an increasing amount of virus particles in diagnostic specimens, development of resistance is the most likely explanation, which therefore, in most cases, requires switching therapy without further diagnostics. In certain patient groups where doubt is raised concerning neuraminidase inhibitor (NAI) levels in the lungs, such as extracorporeal membrane oxygenation patients or patients who received renal support, rapid assays detecting antiviral-resistant viruses are required. Also, in vulnerable patient groups, such as transplant patients, institution of salvage therapy with zanamivir may pose problems with side effects (especially renal problems), method of dosing (inhalation vs intravenous use) and shortage in supply of the intravenous dosage form.

EXPERT REVIEWS

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Generating knowledge on possible resistance in a timely manner may, therefore, benefit patient management. Phenotypic antiviral assays, such as MuNANA and NA-star, determine the susceptibility of influenza viruses to neuraminidase inhibitors by testing the activity of the viral enzyme neuraminidase under increasing inhibitor concentrations [2]. When knowledge on genotypic changes related to phenotypic resistance are lacking due to the introduction of a new neuraminidase inhibitor or influenza subtype, these assays generate valuable information needed for patient management. However, for routine patient management, they are not suitable, since these assays require very high viral load input and, therefore, often a preparatory time-consuming virus-culture step. If sufficiently high viral loads are present in a patient sample, direct phenotypic resistance analysis interpretation of results is inherently difficult. Genotypic resistance assays include traditional Sanger-sequencing, pyrosequencing and real-time RT-PCRs. Conventional bulk Sanger sequencing requires too much time to generate results, and is not sensitive for minority-resistant species in a quasispecies [3].

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Rapid single-nucleotide polymorphism detection techniques that are able to detect mutations at the main NAI resistance sites have been developed. These include pyrosequencing and real-time RT-PCR assays based on minor groove binder (MGB) locked nucleic acid (LNA) probes or melting-curve analysis [4,5]. Pyrosequencing-based assays are, however, highly contamination sensitive, and require instrumentation not present at many diagnostic laboratories. Real-time RT-PCR assays based on MGB/LNA probes are rapid and can be run in multiplex with the screening real-time RT-PCR if designed properly. In a significant percentage of the patients treated with NAI that develop resistant strains, wild-type and resistant mutant strains are detected as mixtures [M. Schutten, Unpublished Data]. In such mixtures, the resistant mutant may not be detected using traditional Sanger sequencing. Genotyping techniques, such as pyrosequencing and MGB/LNA or melting-curve analysis real-time RT-PCRs may, therefore, be more applicable for patient management. MGB/LNA and melting-curve analysis real-time RT-PCRs may be sensitive to mutations in the probe region that are not related to NAI resistance and, therefore, need specialist interpretation.

Only little experience is present on the proper interpretation of data generated with these relatively new techniques to the management of suspected cases. Historically, institution of hygienic control measures and antiviral therapy for influenza is made on data generated with rapid tests. Especially, the higher sensitivity of real-time RT-PCRs poses significant problems with respect to interpretation toward patient management. At our institute, we decided to act on positive results with Ct values below 35 (viral load ~500 viral particles/ml) and classify samples with lower viral loads as boarder-line positive requiring an additional sample with a higher viral load from the same patient for institution of hygienic measures or therapy changes. Such interpretation algorithms are, however, completely based on expert opinion and lack the support of clinical data.

In modernly equipped and organized virus diagnostic laboratories, real-time RT-PCR results on diagnostic samples can be generated within 5–24 h after receipt of the sample at the laboratory. Most clinical decisions regarding institution of therapy and containment of suspected cases are made within hours after presentation of a patient to the clinic. Only when quarantine is capacity limited or serious adverse advents of NAI treatment are expected from antiviral drugs (e.g., patients with renal problems) a clinician is willing to wait for a real-time RT-PCR result if a rapid-test result is inconclusive. Most molecular diagnostic results are, therefore, at best used to adjust decisions already made. The first phase of the MexFlu pandemic, therefore, highlights the need for point-of-care devices for the diagnosis of respiratory infections.

Lessons learned from the pandemic

Despite the relatively mild nature of the first phase of the MexFlu pandemic, it seemed that the whole system – from development of new tests, distribution of essential materials for inhouse-developed tests (plastics and kits for semi-automated nucleic acid-extraction stations and mastermixes for real-time RT-PCRs) and commercial tests, to clinical management of diagnosed patients – was almost overwhelmed. US FDA-approved and CE-marked tests were available relatively quickly but not readily available in sufficient amounts where needed. Well-validated inhouse-developed tests were also available relatively rapidly but distribution of protocols and updates of protocols and controls was slow and uncoordinated. Distribution of protocols and positive controls on a global scale depended on existing structures and individual specialized laboratories and, in only a few countries, such as The Netherlands [6,7], well-organized structures were present to coordinate the diagnostic need. Distribution of protocols through publication in international literature is, however, too slow and may not reach laboratories that do not have subscription to journals willing to rapidly publish. Individual specialized laboratories willing to distribute positive controls can be easily overwhelmed by the number of global requests. The international response seemed adequate, but did not give high hopes for a well-organized response to a truly serious threat, such as the Spanish Flu pandemic in 1918.

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Therefore, it seems imperative that structures are implemented on a global scale for the rapid response to potential rapid global health threats. Coordination by experts specialized in microbiological diagnostics, commercial enterprises working on development of diagnostic kits and structures such as the WHO, US CDC, ECDC, European Society for Clinical Virology, Pan American Society for Clinical Virology, European Network for

Diagnostics of 'Imported' Viral Diseases and Quality Control for Molecular Diagnostics organized into one global response network would benefit future diagnostic responses. One could argue that, during the last 10 years, human society has had sufficient warning signs (1998: H5N1; 2003: SARS; 2005: Chicungunya; 2008: oseltamivir-resistant H1N1; and 2009: MexFlu) to start acting upon.

Financial & competing interests disclosure

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