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Viral aetiology of acute respiratory illnesses in patients with suspected severe acute respiratory syndrome (SARS) in Switzerland

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Most acute viral respiratory illnesses share clinical features with severe acute respiratory syndrome (SARS) and detection of the SARS-associated coronavirus may be negative in the course of the disease. It is thus of interest to identify other viral infections to ensure a better assessment of the epidemiological situation and avoid unnecessary isolation and contact tracing.

Patients considered potential SARS cases in Switzerland were tested systematically for ten different respiratory viruses. RT-PCR for the SARS-associated coronavirus was negative in all cases. In contrast, other respiratory virus infections were identified in 62% of cases (21/34). Influenza was the most frequent, being recovered in 14 cases (41%); other viruses were rhinovirus, respiratory syncytial virus, parainfluenza, human metapneumovirus and adenovirus. A negative coronavirus RT-PCR, together with a positive result for another respiratory virus and a consistent clinical course, made it possible to avoid unnecessary isolations and contact tracing. Our findings are also consistent with the absence of large outbreaks of SARS in Europe during this period.

The World Health Organisation (WHO) has provided a case definition for severe acute respiratory syndrome (SARS) based on travel history (or contact with a known SARS case) and a spectrum of clinical symptoms in persons presenting with acute febrile respiratory illness [1, 2]. It is within the aims of this definition to be highly sensitive and thus allow prompt identification of cases. However, due to its lack of specificity this strategy has several limitations and may result in an inaccurate description of the epidemi-

logical situation, particularly in low-prevalence countries. Indeed, many common acute viral respiratory illnesses, such as those caused by influenza, respiratory syncytial virus, parainfluenza or human metapneumovirus, share clinical features with SARS. Furthermore, the spectrum of the disease following SARS-associated coronavirus infection is not yet fully established, and self-limited respiratory tract illnesses not fulfilling WHO criteria are likely to occur [3–5]. Methods for the detection of SARS-associated coronavirus have only been partially validated and RT-PCR specific assays may be negative in the course of the disease [6]. For these reasons it is important to diagnose other viral aetiologies in patients with suspected SARS, to avoid both unnecessary investigations and quarantine. We present here the results of virological investigations in potential SARS patients in Switzerland.

Patients identified as potential SARS cases according to WHO criteria, and those not fulfilling all the criteria but presenting with acute respiratory illness and either a recent travel history in a country or in an affected area or a documented contact with a suspected SARS case, were tested for a wide range of viruses in upper respiratory tract samples (nasopharyngeal swabs). Nasopharyngeal specimens were inoculated for virus culture on 6 different cell lines (human fibroblast, A549, MDCK, LLC-MK2, Rita and Vero cells) in tubes and incubated at two different temperatures (37 °C and 33 °C).

RNA was extracted from 200 µl of each specimen using 400 µl lysis buffer (HCV Amplicor Specimen Preparation Kit, Roche Diagnostics Corporation, Indianapolis, ID). Retrotranscription was performed using the SuperscriptTM II RNase H⁻ Reverse Transcriptase (Invitrogen, Switzerland) and random primers (dN)₆ (Roche Diagnostics GmbH, Germany) for 60 minutes at 42 °C. Real-time Taqman[®] PCR (ABI Prism[®] 7900 HT, Applied Biosystems, Switzerland) was performed for detection of respiratory viruses using specific primers and probes validated in our laboratory. Screening for the presence of the following viral RNA genomes was conducted (target gene in brackets): influenza A (M gene) and B (haemagglutinin gene), respiratory syncytial virus A and B (N gene), parainfluenza 1 and 2 (haemagglutinin-neuraminidase gene), human metapneumovirus (polymerase gene), rhinovirus (5' non-coding-region) and enterovirus (5' non-coding-region), and the SARS-associated coronavirus (polymerase gene). The following primers and probe were used for SARS-associated coronavirus detection: forward 5'-TCACC-GCGAAGAAGCTATT-3'; reverse AGTT-GCATGACAGCCCTCTACA-3'; and probe 5'-CGTTCGTGCGTGGATTGGCTTT-G-3' based on available sequences of the polymerase gene [7, 8]. In preliminary experi-

ments this assay proved to detect fewer than 10 RNA copies/µL. Positive and negative controls were included in each run.

In Switzerland during the first six weeks of surveillance following the WHO SARS alert, we identified 34 subjects with a median age of 36 years (range 2–81), comprising 20 males and 14 females. On the basis of WHO criteria, three cases were probable SARS, 16 were suspect cases, and in 15 the diagnosis of SARS was considered but the WHO criteria were not met. All patients recovered without suffering severe disease. RT-PCR for detection of the SARS-associated coronavirus proved negative in all subjects (median day 4 of illness, range 1–9). In contrast, other respiratory virus infections were identified in 62% (21/34). Among the 19 suspected or probable SARS cases according to WHO criteria, a virus was identified in 11 (59%). In the overall population influenza viruses were identified in 14 cases (41%), rhinovirus in 3, respiratory syncytial virus in 1, parainfluenza 1 in 1, human metapneumovirus in 1 and adenovirus in 1. RT-PCR for enterovirus and parainfluenza 3 were all negative. Among the 21 positive cases, 11 were identified by RT-PCR only, two by cell culture only (parainfluenza 1 and adenovirus), and the remaining 8 by both methods concomitantly.

Our study showed that all subjects with suspected SARS in Switzerland tested negative for the SARS-associated coronavirus by RT-PCR. However, following extensive testing, a viral aetiology was documented in the majority of cases. Although a viral aetiology was likely in most of the remaining cases, we failed to identify a virus. This limited sensitivity was related to several reasons including the delay between the onset of symptoms and the time of sampling, and the type of the specimen.

As some recent observations suggest that RT-PCR may be negative in the course of SARS [6, 9], it is essential to provide an alternative diagnosis to SARS if a negative result is obtained. In our series a negative RT-PCR together with a positive result for another respiratory virus and a consistent clinical course made it possible to avoid unnecessary isolations, investigations and contact tracing. In addition, in some instances suspicion of local secondary transmission of SARS could be ruled out when influenza was documented in the index case. The epidemiological situation could be better evaluated since the number of cases remaining suspect for SARS was considerably reduced. Influenza virus was the most frequent cause of respiratory illness in this population, an observation consistent with the fact that the SARS alert coincided with the peak of the influenza outbreak in Switzerland. However, since the majority of cases reported recent travel to Asia, this observation also demonstrates the risk of acquiring influenza and other respira-

tory viruses abroad and their capacity for subsequent transmission locally. Finally, our findings are consistent with the observation that during this period SARS did not cause serious outbreaks in Europe.

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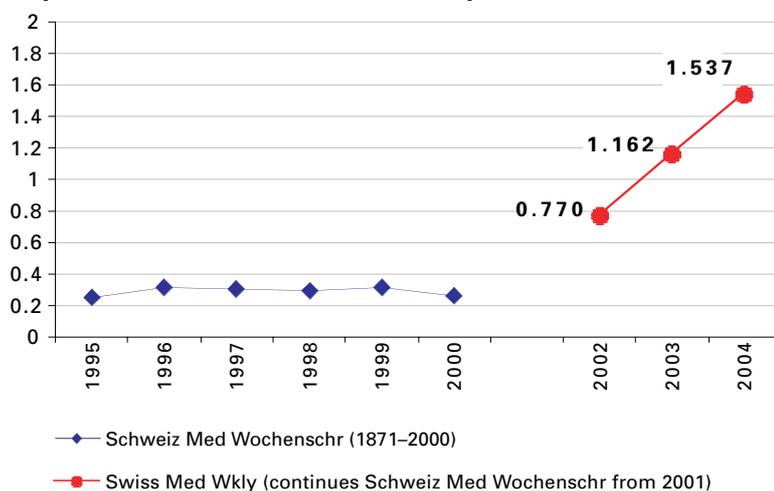
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