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Clinical symptoms cannot predict influenza infection during the 2013 influenza season in Bavaria, Germany

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SUMMARY

For influenza surveillance and diagnosis typical clinical symptoms are traditionally used to discriminate influenza virus infections from infections by other pathogens. During the 2013 influenza season we performed a multiplex assay for 16 different viruses in 665 swabs from patients with acute respiratory infections (ARIs) to display the variety of different pathogens causing ARI and to test the diagnostic value of both the commonly used case definitions [ARI, and influenza like illness (ILI)] as well as the clinical judgement of physicians, respectively, to achieve a laboratory-confirmed influenza diagnosis. Fourteen different viruses were identified as causing ARI/ILI. Influenza diagnosis based on clinical signs overestimated the number of laboratory-confirmed influenza cases and misclassified cases. Furthermore, ILI case definition and physicians agreed in only 287/651 (44%) cases with laboratory confirmation. Influenza case management has to be supported by laboratory confirmation to allow evidence-based decisions. Epidemiological syndromic surveillance data should be supported by laboratory confirmation for reasonable interpretation.

Key words: Influenza, influenza (seasonal), public health, public health microbiology, respiratory infections.

INTRODUCTION

Acute respiratory infections (ARIs) are a leading cause of morbidity. Influenza viruses alone were responsible for an estimated number of 7·7 million excess consultations and 32 000 hospitalizations during the 2013 influenza season in Germany [1]. As a consequence surveillance systems are in place to monitor the onset of influenza season, and to provide epidemiological data for clinical decision making. Most influenza surveillance systems use syndromic case definitions [2–4], i.e. the WHO case definitions for ARI and/or influenza-like-illness (ILI) [5]. In addition to syndromic surveillance some countries collect virological data from swabs of ARI/ILI patients [1] to describe circulating virus strains and to gather data for next season's influenza vaccine [2–4]. Besides these data on seasonal influenza circulation, physicians also rely on typical symptoms of an influenza infection: sudden onset, cough, high temperature and general malaise to reach a diagnosis.

However, mild courses of influenza infection do occur [6, 7] and cannot be distinguished from ARIs caused by other viruses. For example, infections of the respiratory tract by adenoviruses or respiratory

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syncytial virus (RSV) often resemble influenza and it is widely accepted that symptoms of viral respiratory tract infections alone are rarely indicative of a single pathogen [8]. Therefore, both clinical and epidemiological case definitions as well as the individual physician's judgement might miss true influenza cases.

During an influenza season the positivity rate for influenza viruses in swabs, i.e. laboratory-confirmed cases, may reach up to 60–70% [1]. Although virological surveillance systems are an asset in the monitoring of influenza epidemics, these figures also emphasize that a considerable proportion of ARI/ILI patients who are swabbed during an influenza season are infected by other pathogens [9].

In order to display the variety of viruses which cause ARIs and especially ILI during an influenza season, and to test the diagnostic value of symptoms-based case definitions for a correct influenza diagnosis, we screened swabs of ARI patients for 16 different viruses during the 2013 influenza season. Additionally, we aimed to estimate the positive predictive value of the clinical diagnosis 'influenza', i.e. the physician's judgement, through a questionnaire survey of the participating physicians.

MATERIALS AND METHODS

Specimen sampling

During the 2013 influenza season (calendar weeks 3–14) nasal or pharyngeal swabs from ARI patients were collected by two paediatricians and two general practitioners who took part in the Bavarian Influenza Sentinel (BIS) surveillance. BIS was implemented in 2009 by the Bavarian Health and Food Safety Authority (LGL) to supplement syndromic surveillance with virological data [10]. Sampling of swabs began when virological surveillance systems [2] noted a positive rate >20% of influenza-positive swabs within the sentinel and was limited to 12 weeks. The physicians had the possibility to swab up to 20 different ARI patients every week. On Monday or Tuesday of each week, swabs were taken from consecutive ARI patients with a Sigma-Swab® in Sigma-Virocult® media [both Medical Wire & Equipment Co. (Bath) Ltd, UK]. Samples were sent together with a patient questionnaire to the LGL for laboratory confirmation of a viral infection. Each patient was swabbed once only.

Epidemiological investigations

Using a pre-tested structured questionnaire, the physicians reported for each patient, age, clinical symptoms (time of onset, temperature, cough) and if the patient was assessed to have influenza or not based on his/her clinical experience. Age groups were defined (0–4, 5–14, 15–34, 35–59, $\geqslant$60 years).

ARI was defined as acute onset if at least one of the following symptoms were present: cough, sore throat, shortness of breath and coryza with or without fever. Additionally, a physician's judgement was required confirming that the illness was due to an infection. ILI was defined as ARI with onset during the last 7 days with a measured temperature of $\geqslant$38 °C and additional cough [5]. Whereas all cases were regarded as ARI cases (inclusion criterion), ILI cases were deduced from the information on the questionnaires and laboratory-confirmed cases were defined by influenza RNA detection.

Virological analysis

The samples were tested for 16 different viruses [adenovirus, coronavirus (NL63, OC43, HKU1, 229E), enterovirus/rhinovirus, human metapneumovirus, human bocavirus, influenza virus type A (subtype H1N1, subtype H3), influenza virus type B, parainfluenza virus (PIV-1, PIV-2, PIV-3, PIV-4), respiratory syncytial virus (RSV)] using the Luminex xTag RVP (respiratory virus panel) assay (Luminex, Molecular Diagnostics, Canada) according to the manufacturer's instructions [11]. Total nucleic acid was extracted from the swabs using the QIAamp Virus BioRobot 9604 kit (Qiagen, Germany), employed on a Microlab Star Hamilton robot (Hamilton Robotics GmbH, Germany). PCR was performed on an Eppendorf Mastercycler Nexus (Eppendorf, Germany) and hybridization/identification carried out on a Bio-Plex MAGPIX Multiplex Reader (Luminex).

Data were analysed using Stata v. 12.1 software (StataCorp., USA) and χ^2 test.

RESULTS

Detection of viral RNA and DNA in swabs

In total, 665 swabs from ARI patients were collected and viral RNA or DNA was detected in 484 (73%) samples. Due to co-infections 558 viruses were identified in these samples. The majority of cases were infected by influenza A virus (28%), influenza B virus (16%), RSV (15%), enterovirus/rhinovirus (10%) and coronavirus NL63 (5%). Other respiratory viruses were detected at lower frequencies (Fig. 1).

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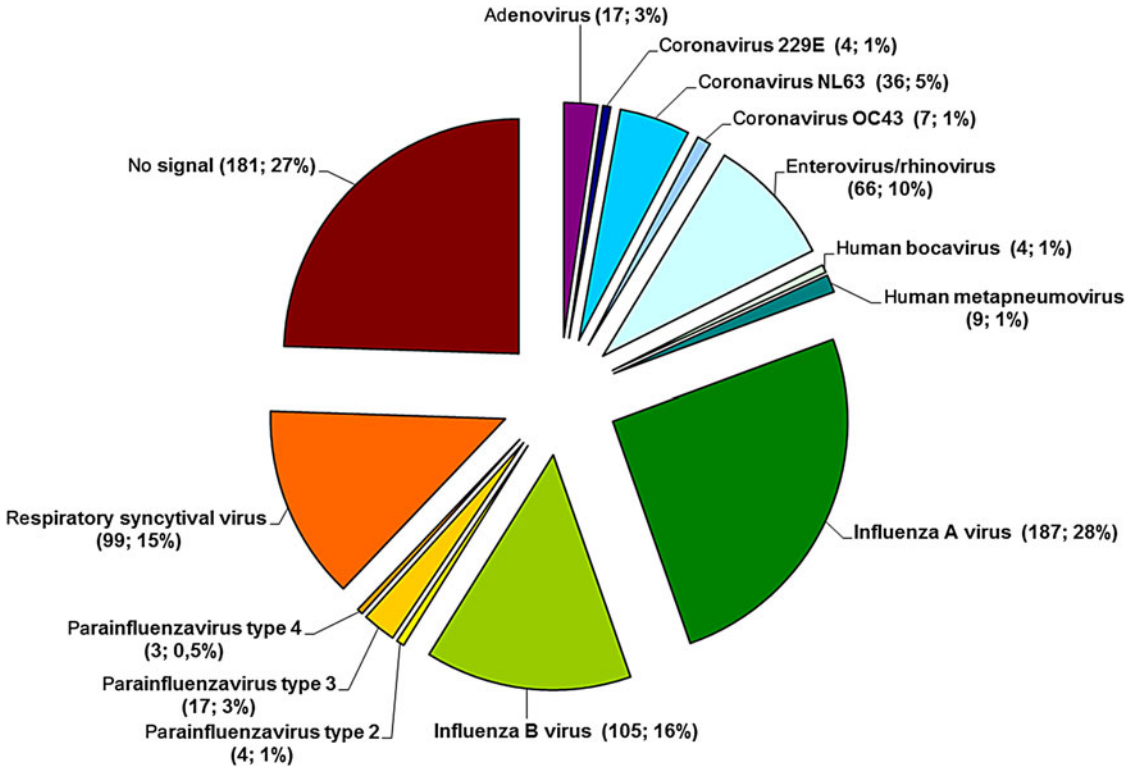

Fig. 1. Detection of viral RNA or DNA in swabs from 665 patients with acute respiratory infections in calendar weeks 3–14 (2013). Values in parentheses are number of cases and percentage of each virus relating to 665 swabs.

Influenza positivity rate peaked in calendar week 5 (83/144, 58%). One hundred and eighty-one (27%) samples tested negative for viruses covered by the the Luminex assay. Co-infections were observed in 58/655 (9%) swabs, most commonly enterovirus/rhinovirus and RSV ($n = 13$) followed by enterovirus/rhinovirus and influenza ($n = 11$) and influenza and RSV ($n = 10$).

Coronavirus HKU1 and PIV-1 were not detected by the RVP assay during the observation period.

Age-related viral diversity

Viral infections were detected in 89% (144/161) of ARI patients in the youngest age group ($P < 0{\cdot}001$ compared to the other age groups), in 66% (118/180) of the 5–14 years age group, in 69% (90/131) of the 15–34 years age group, in 68% (68/164) of the 35–59 years age group and in 68% (19/28) of the $\geqslant 60$ years age group. It should be noted that in the latter group only 28 samples were available.

Influenza viruses, RSV, enterovirus/rhinovirus and coronavirus NL63 were detected in every age group. The diversity of detected viruses was highest in the youngest ($n = 12$) and lowest in the oldest ($n = 5$) age groups, respectively (Fig. 2).

Twenty-nine per cent (47/161) co-infections with 2–4 different viruses were identified in the youngest age group (0–4 years), 7% (13/180) in the 5–14 years age group, 6% (8/131) in the 15–34 years age group and 4% in both the 35–59 years (6/164) and the $\geqslant 60$ years (1/28) age groups.

In the youngest age group RSV was most often detected (61/161, 38%), followed by influenza A/B (29%; A: 31/161, 19%, B: 15/131, 9%) and enterovirus/rhinovirus (33/161, 20%). In the 5–14 years age group, influenza B virus (52/180, 29%) was more frequent than influenza A virus (40/180, 22%). Likewise, in the 15–34 years age group, influenza A virus was most often detected (40%, 52/131), and also present in 35% (57/164) of samples in the 35–59 years age group; influenza B virus was also detected in 15% (24/164) of this age group.

In the oldest age group RSV was found as often as influenza A virus (7/28, 25%) RSV infections showed a bimodular pattern with highest detection rates in the youngest and the oldest age groups (Fig. 2).

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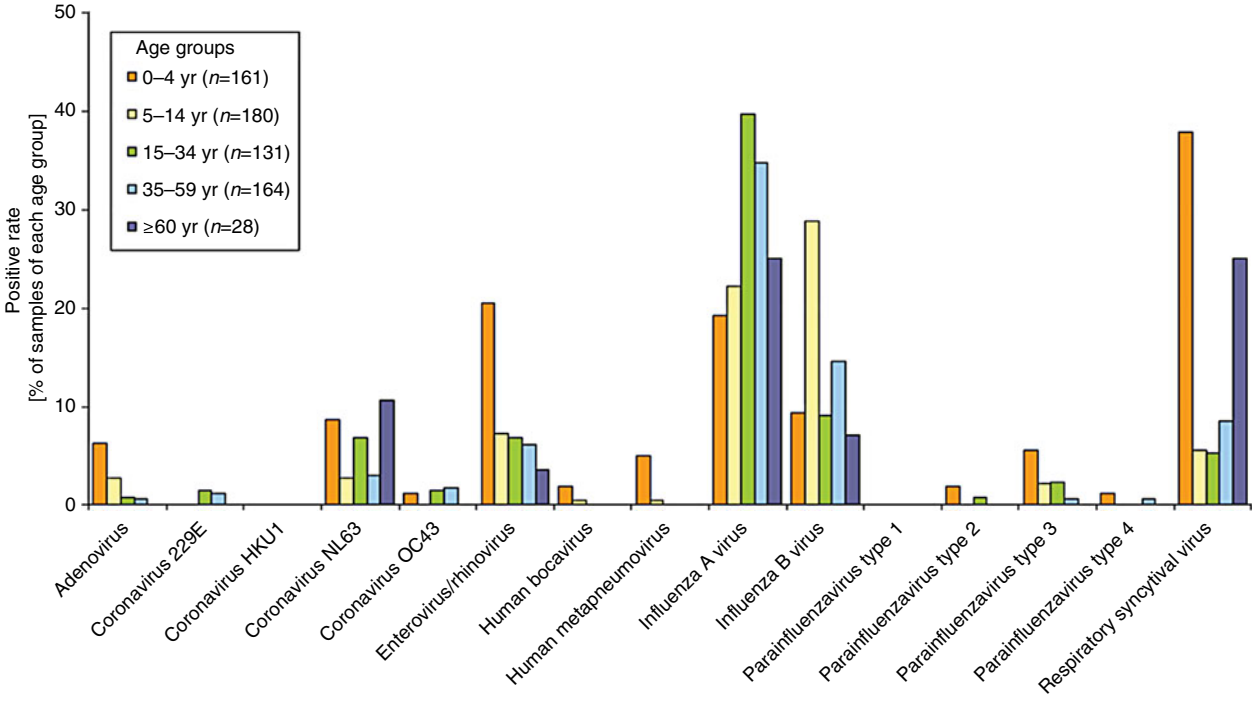

Fig. 2. Percentage of infections with different viruses in different age groups in swabs from 665 patients with acute respiratory infections in calendar weeks 3–14 (2013).

Laboratory-confirmed virus detection in ILI patients

The ILI definition (ILI+) was met by 431/665 (65%) of ARI patients. Influenza virus infection was confirmed in 215/431 (50%) by RNA detection, showing that the ILI definition significantly overestimates the number of patients with influenza infection ($P < 0{\cdot}001$).

In 100/216 (46%) patients that fulfilled the ILI criteria (ILI+) and whose expected influenza infection was not laboratory confirmed (RNA-), no other viral infection was detected by the RVP assay. In the remaining 116 (54%) patients, including co-infections, viruses other than influenza were detected on 143 occasions: RSV ($n = 60$) was most frequently detected followed by enterovirus/rhinoviruses ($n = 34$), coronavirus NL63 ($n = 14$) and adenovirus ($n = 13$) (Table 1). The ILI definition (ILI-) was not fulfilled by 234/665 (35%) ARI patients. Laboratory-confirmed influenza (RNA+) was detected in 76/234 (33%) of these patients.

Laboratory-confirmed virus detection in patients with assumed influenza

Questionnaire analysis revealed that 409/651 (63%, no influenza assessment in 14 patients) patients were expected by their respective physicians to be confirmed as 'true' influenza patients according to their clinical judgement (Flu+). However, influenza virus infection was confirmed only in 214/409 (52%) by RNA detection.

In 118/195 (61%) patients without laboratory-confirmed influenza (RNA-) no other viral infection was detected by the RVP assay. The remaining 77/195 (39%) RNA- patients were infected by other pathogens. Due to 11 co-infections, 94 viruses were identified in the samples. RSV ($n = 37$) and enterovirus/rhinoviruses ($n = 22$) were most frequently detected (Table 1). In 242/651 (37%) patients the physician did not expect their patient to be infected by influenza virus (Flu-). However, in 64/242 (26%) of those patients influenza RNA was detected.

Statistical analysis shows that the physician's assessment of an ILI significantly differs from the laboratory confirmation of an influenza infection ($P < 0{\cdot}001$), indicating that physicians overestimated the number of ILI cases.

In 176 influenza RNA-positive and in 111 influenza RNA-negative cases both ILI definition and the physicians' assessment of influenza were concordant with their laboratory testing results, respectively. Therefore, 176 people were labelled as RNA+, ILI+, Flu+ and 111 as RNA-, ILI-, Flu-. Taken together, the ILI definition, the physicians' assessment and the

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Table 1. *Detection of different respiratory viruses in influenza negative (RNA-) patients who fulfil the influenza-like illness definition (ILI+) or in which influenza was assumed by the physician (Flu+)*

	ILI+ RNA-		Flu+ RNA-	
	No. of infections	% infected	No. of infections	% infected
Respiratory syncytial virus	60	42	37	39
Enterovirus/rhinovirus	34	24	22	23
Coronavirus NL63	14	10	9	10
Adenovirus	13	9	9	10
Parainfluenzavirus type 3	8	6	7	7
Human metapneumovirus	5	3	2	2
Coronavirus OC43	3	2	2	2
Human bocavirus	3	2	1	1
Coronavirus 229E	2	1	3	3
Parainfluenzavirus type 2	1	1	2	2
Total	143	100	94	100

laboratory test results were concordant in 44% of all tested participants (287/651). The majority of cases (364/651, 56%) were not assigned to the same groups.

Prediction of influenza infection by ILI definition and influenza assessment

ILI definition and influenza assessment by the physicians proposed 431 and 409 influenza cases, respectively, more than those confirmed by RNA detection ($n = 291$). The sensitivity of ILI definition and influenza assessment was 74% and 77%, and specificity was 42% and 48%, respectively. The positive predictive value (PPV) of the ILI definition for laboratory-confirmed influenza was 50% and the negative predictive value (NPV), 68%. Likewise, the PPV of the influenza assessment confirmed by the laboratory was 52%, and the NPV, 74% (Table 2).

Table 2. *Sensitivity, specificity, positive predictive and negative predictive values of influenza-like illness definition (ILI+/-) and influenza assessment (Flu+/-) vs. laboratory confirmation of influenza viruses (RNA+/-)*

	RNA +	RNA -	Total		
ILI +	215	216	431	**PPV**	**50%**
ILI -	76	158	234	**NPV**	**68%**
Total	291	374	665		
	Sensitivity 74%	**Specificity 42%**			
	RNA +	RNA -	Total		
Flu +	214	195	409	**PPV**	**52%**
Flu -	64	178	242	**NPV**	**74%**
Total	278	373	651		
	Sensitivity 77%	**Specificity 48%**			

PPV, Positive predictive value; NPV, negative predictive value.
Swabs from 665 patients with acute respiratory infections in calendar weeks 3–14 (2013) were investigated.

DISCUSSION

We have attempted to display the variety of ILI-causing viruses during a single influenza season. Moreover, we aimed to test the diagnostic value of both the commonly used ARI and ILI case definitions as well as the clinical judgement of physicians, respectively, to achieve a laboratory-confirmed influenza diagnosis.

A limitation of the study might be a possible heterogeneity of clinical skills in assessing influenza in our small group of physicians. However, we chose long-term collaborators of the BIS with wide experience in clinical influenza diagnosis. Therefore, we regarded them as sufficient for the purposes of our study.

We have demonstrated the co-circulation of a wide variety of different viruses during the 2013 influenza season. The positivity rate of influenza, the plethora of detected viruses and co-infections found especially in the youngest age group, the dominance of influenza B virus infections in the 5–14 years age group and the high infection rate with RSV in the youngest as well as in the elderly are in line with published epidemiological data [12–14] and support the conclusion that

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syndromic surveillance data for influenza based on the ARI or ILI definitions need to be interpreted with care.

The influenza positivity rate of the whole study population was 44%. Besides influenza viruses, a number of other pathogens – primarily RSV, enteroviruses/rhinoviruses, and coronaviruses – were found. These viruses were detected in the same frequency in the ILI+/RNA- group and in the Flu+/RNA- group. Therefore the PPV for a supposed influenza infection based either on the ILI definition or on the physician's clinical judgement was as low as 50% and 52%, respectively.

Both ILI definition and physicians' clinical judgement overestimated the number of influenza infections during the influenza season. This is illustrated by a high false-positive rate and the poor PPV in the ARI population despite the ongoing influenza season. Only for the guidance of sampling for virological surveillance data does the sensitivity of 74% of the ILI definition appear to be acceptable. Taken together, these data suggest a small chance above random to find an influenza infection based on presumed typical symptoms during an influenza season.

Our data underline the importance of laboratory-based viral surveillance to avoid a high rate of both false-positive and false-negative influenza estimates or diagnoses and to identify the ARI- or ILI-causing pathogen(s) [15]. However, in most ARI/ILI cases, laboratory identification is not routinely performed, owing to the lack of specific treatment available for most of the viral respiratory tract infections. Nevertheless, evidence is growing that knowledge of the causative pathogen might have considerable influence on the case management, especially in children, where the specific detection of a viral pathogen may avoid overtreatment with antibiotics [16]. Interestingly, the traditional argument for prescribing antibiotics to prevent possible superinfections is not supported by a recent meta-analysis [17]. Multiplexing assays have become more sensitive and also cheaper in recent years and thus diagnosis and management of respiratory infections should increasingly be based on laboratory evidence as clinical signs and symptoms are rather unreliable for differentiating pathogens.

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DECLARATION OF INTEREST

None.

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