Clinical diagnosis of influenza virus infection: evaluation of diagnostic tools in general practice

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SUMMARY

Background: *With the development of new antiviral agents for influenza, the urge for rapid and reliable diagnosis of influenza becomes increasingly important. Respiratory virus infections are difficult to distinguish on clinical grounds. General practitioners (GPs) however, still depend on their clinical judgement.*

Aim: *To evaluate the importance of clinical symptoms in the diagnosis of influenza virus infection.*

Design of study: *A multicentre questionnaire study.*

Setting: *Eighty-one patients from 14 general practices.*

Method: *Patients with fever and at least one constitutional symptom and one respiratory symptom were included. A questionnaire with the medical history and clinical symptoms was completed and a combined nose–throat swab was taken. Virus culture, rapid culture, and polymerase chain reaction (PCR) amplification were performed on each specimen. Multivariate analysis was used to obtain the best predictive model.*

Results: *By using PCR, an increase was seen in the detection of the viral pathogens compared with the results of culture. In 42 out of 81 patients PCR was positive for influenza. A positive predictive value (PPV) of 75% was observed for the combination of headache at onset, feverishness at onset, cough, and vaccination status during the period of increase influenza activity. Criteria used by the ICHPPC-2 resulted in a PPV of 54%. The PPV for diagnosis made by the GP was 76%.*

Conclusion: *Although influenza is difficult to diagnose on clinical grounds, the GPs in this study were able to diagnose influenza as such more accurately on their judgement than by the other criteria.*

Keywords: *influenza; clinical diagnosis; antiviral drugs; polymerase chain reaction; virus culture; rapid culture.*

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Introduction

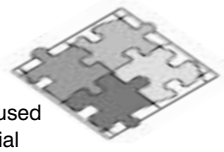
EACH year the general practitioner (GP) is confronted with the seasonal, local, and/or regional influenza epidemic. Although the impact and complications of influenza virus infection are well known,[1] active policy by the GPs is limited by the yearly vaccination of people at risk. The problems that are encountered by the GP are the difficulty in distinguishing influenza virus infection clinically from other respiratory infections, the lack of rapid laboratory diagnostic tools, and the limited possibilities for intervention.

Recently, promising results have been published of trials with new antiviral compounds, the neuraminidase inhibitors, which are effective against influenza A and B.[2-5] They are to be taken within 48 hours of infection to be effective. Two of these agents, zanamivir and oseltamivir, have recently been registered in some countries for treatment. With the development of these new treatment options, rapid diagnosis gains relevance for GPs. In the absence of laboratory tests that are feasible, reliable, and rapid, influenza diagnosis still has to be made by evaluation of signs and symptoms.

In this study, we evaluate the importance of clinical symptoms to diagnose influenza virus infection for GPs. Most studies so far have focused on hospitalised children or the elderly, either emphasising severe symptoms or lack of symptoms.[6-9] Although it is difficult to identify influenza based on clinical characteristics, diagnostic criteria have been formulated. The criteria to differentiate between influenza virus infection and infection caused by other respiratory viruses are not uniform. Guidelines for the diagnosis of influenza are formulated for GPs through the criteria of the International Classification of Health Problems in Primary Care (ICHPPC-2). Influenza is diagnosed when there is an influenza outbreak and a patient has four of the following symptoms: sudden onset, contact with influenza, fever, cough, chills, malaise, myalgia, hyperaemic mucous membranes of the nose and throat, or six of these symptoms outside an influenza outbreak.[10] The Netherlands Institute of Primary Health Care (NIVEL) is running a registration network of 46 sentinel general practices spread over the country. The NIVEL reports patients with acute respiratory illnesses. They define influenza-like illness (ILI) as abrupt onset (prodromal phase with minor symptoms of less than five days), rectally measured body temperature of at least 38ÞC and at least one of the following symptoms: cough, coryza, headache, retrosternal pain or myalgia.[11]

The aim of this study was to investigate the relationship between signs and symptoms and the presence of influenza virus infection and to assess the accuracy of the clinical diagnosis by GPs in patients with an acute respiratory ill-

HOW THIS FITS IN



What do we know?
Despite yearly vaccination, infections caused by influenza viruses still lead to substantial morbidity and mortality. Distinguishing influenza clinically from other respiratory viruses is difficult. Formulated guidelines to diagnose influenza do not distinguish satisfactorily.

What does this paper add?
Notification of the results of surveillance networks to general practitioners and their interpretation of presenting symtoms are important tools for diagnosing influenza.

ness. To evaluate the clinical presentation we have chosen to use the more sensitive PCR besides virus culture and antigen testing. The ICHPPC-2 criteria, the sentinel criteria, and the results of our clinical questionnaire were matched with the results of the most sensitive laboratory technique.

Method

Study design

From November 1997 to May 1998, 14 GPs in the Utrecht region in the Netherlands included patients who presented at their practice with: fever (≥38°C, anamnestic), at least one constitutional symptom (malaise, headache, myalgia, chills), and at least one respiratory symptom (coryza, sneezing, cough, sore throat, hoarseness). Patients were asked to participate when these symptoms existed for ≤48 hours. A physical examination was carried out by the GP and a questionnaire was completed. A combined nose and throat swab was taken for the laboratory detection of virus. The questionnaire contained the following items: inclusion criteria, administrative data (initials, date of birth, sex), medical history, medication, smoking habits, influenza vaccination status, presenting symptoms, contact with other patients with similar symptoms, onset of symptoms, physical examination, therapy, and the presumed aetiology of illness by the GP prior to the results of the virological diagnosis.

The NIVEL criteria for increased influenza activity were used: increased influenza activity means that the threshold of 5/10 000 inhabitants with ILI is exceeded. An influenza epidemic is spoken of when the threshold exceeds 40/10 000 inhabitants with ILI.[12]

Virological methods

Nose and throat swabs were obtained for virus isolation and either transported to the laboratory in virus transport medium directly or stored at 4°C for a maximum of 24 hours at the general practice. Part of the patient material was used for immediate culture of influenza viruses, parainfluenza viruses, picornaviruses, RSV, adenoviruses, and herpesviruses. After two days of culture, rapid antigen testing was performed by immunofluorescence with monoclonal antibodies for influenza viruses, parainfluenza viruses, RSV, and adenovirus (rapid culture).

The remaining material was frozen and stored at -70°C for later analysis. On the remaining material polymerase chain reactions (PCR) were performed for influenza A and B virus, parainfluenza virus 1, 2, and 3, picornaviruses (rhinovirus and enterovirus), respiratory syncytial virus (RSV), and coronaviruses. Rhinoviruses were identified by *Bgl I* digestion of the picornavirus RT-PCR amplicons.[13] Viral nucleid acid extraction was performed according to the method of Boom *et al*.[14] For all PCR reactions a one-tube reverse transcriptase polymerase chain reaction (RT-PCR) was followed by a nested polymerase chain reaction (nested-PCR). Similar RT-PCR and nested-PCR conditions were used as described by Nijhuis *et al*.[15]

Statistical analyses

Data were analysed by χ^2 or Fisher's exact test. Logistic regression was used for multivariate analysis, using all relevant patient characteristics and influenza symptoms at onset and at presentation ($P<0.15$ in the univariate analysis) as independent variables and positive PCR result for influenza A or B as a dependent variable. $P<0.05$ was considered significant.

Results

Patient population

A total of 81 patients were included by 14 GPs from 1 November 1997 to 1 May 1998. Thirty-three (41%) of the patients were male. Thirty-three patients were aged under 25 years and 43 patients were aged between 25 to 65 years. Only five patients were included over 65 years of age. The majority of patients were otherwise healthy individuals.

Laboratory findings

All of the 33 culture or rapid culture positive samples were tested positive by PCR. In addition, viral pathogens were identified in another 19 patients using PCR. The NT-swabs that were taken of all 81 patients included during the surveillance period yielded 53 pathogens: 42 influenza A viruses, five rhinoviruses, three coronaviruses, two RSV and one adenovirus. No mixed infections were found (Table 1).

Viruses were detected in samples from 26/33 (79%) patients under 25 years of age and 26/43 (60%) patients aged between 25 to 65 years. One out of the five patients over 65 years of age (20%) was found positive by PCR.

Table 1. Various respiratory viruses detected by PCR in NT-swabs of patients presenting with influenza-like illnesses. Values are numbers of samples.

Virus	PCR
Influenza virus A	42
Influenza virus B	-
Picornavirus	5
Respiratory syncytial virus	2
Coronavirus	3
Parainfluenza virus	-
Adenovirus	ND[a]
No virus detected	29
Total	81

[a]Adenoviruses were only diagnosed by culture/rapid culture: cell culture yielded one positive result.

Distribution of infection

The rate of detection of viruses was not equally distributed during the six-month surveillance period: influenza virus A was mainly detected during the end of winter and beginning of spring (February through March 1998). According to the NIVEL surveillance data the influenza season was mild: during weeks 8–14 in 1998 there was increased influenza activity and a maximum of 17/10 000 inhabitants with ILI was seen in week 9.[16] We found that 42/81(52%) patients were indeed infected with influenza virus.

Predictive value of criteria and clinical presentation

Seventy-nine out of 81 received questionnaires could be evaluated. Clinical features of 79 patients with clinical illness during this period were compared with viral detection of influenza virus A by PCR (Table 2). Vaccination for influenza virus was significantly correlated with a negative outcome for influenza virus infection ($P \leq 0.05$). Cough as a presenting symptom was significantly correlated with influenza A virus infection compared with the group of patients of which other respiratory viruses or no viral pathogen could be detected ($P \leq 0.01$, positive predictive value [PPV] = 57%, negative predictive value [NPV] = 90%). Headache at onset of symptoms and feverishness at onset of symptoms were also positively correlated with influenza A virus infection ($P \leq 0.05$, PPV = 71%, NPV = 61% and PPV = 63%, NPV = 61% respectively). No other relations between clinical features and positive PCR could be found. Variables with a $P < 0.15$ (period of increased influenza activity, cough, hoarseness, feverishness, headache at onset of symptoms, chronic obstructive pulmonary disease, vascular disease, and vaccination for influenza virus) were combined in a logistic regression model. Stepwise deletion of variables showed the best model with the combination of period of increased influenza activity, cough, headache at onset, feverishness at onset and vaccination status with a PPV of 75% and a NPV of 80%.

All of the patients met the NIVEL criteria for ILI. Fifty-two per cent (41/79) were infected with influenza virus. Seventy-two of the patients met the criteria of ICHPPC-2. The criteria of ICHPPC-2 showed a PPV of 54% and a NPV of 85% (Table 3). The GPs were asked to fill in their presumed aetiology of illness of the patients (influenza, other respiratory virus, and no viral pathogen). There was a significant correlation between the opinion of GPs and the outcome of pathogen in case of influenza virus infection ($P \leq 0.01$, PPV = 76%, NPV = 75%).

Discussion

The results of our study on the complex of symptoms typical for influenza virus infection demonstrate a positive predictive value of 75% and a negative predictive value of 80% for the combination of cough, headache at onset, feverishness at onset, and vaccination status during the period with increased influenza activity. The GPs opinion on the viral aetiology of infection showed a PPV of 76% and a NPV of 75%. By using PCR an increase is seen in the detection of the viral agents responsible for the symptoms of disease.

Few studies have been done to evaluate the clinical presentation of respiratory virus infection. Govaerts *et al* found in their study on the predictive value of influenza symptomatology in the elderly a predictive value of 44% of the complex of fever, acute onset, and cough.[6] Our study is limited by the small group of patients in different age groups that only represents patients who visit their GP. It is therefore difficult to

Table 2. Clinical findings of patients with ILI according to PCR result for influenza. Values are numbers of patients (percentages) and relative risk (RR) with 95% confidence intervals (95% CI).

Clinical observation	Influenza virus A (n = 40)	Unknown (n = 39)	RR	95% CI
Respiratory symptoms				
Nasal congestion	27 (67.5)	23 (59)		
Sneezing	13 (32.5)	12 (31)		
Cough	39 (97.5)	30 (77)[b,c]	11.7	1.40–97.5
Hoarseness	3 (7.5)	9 (23)[c]	0.3	0.70–1.1
Sore throat	32 (80)	27 (69)		
Shortness of breath	6 (15)	11 (28)		
General symptoms				
Headache	28 (70)	22 (56)		
Feverishness	35 (87.5)	33 (85)		
Myalgia	24 (60)	24 (62)		
Malaise	29 (72.5)	29 (74)		
Symptoms of onset				
Cough	17 (41)	18 (46)		
Sore throat	18 (47)	14 (35)		
Headache	20 (56)	8 (21)[b,c]	3.9	1.40–10.50
Myalgia	12 (28)	8 (20)		
Feverishness	24 (56)	14 (35)[a,c]	2.7	1.10–6.70
Patient characteristics				
Vaccination	1 (2.5)	7 (18)[a,c]	0.1	0.01–1.00
Vascular disease	0 (0)	3 (7.5)[d]		
Diabetes mellitus	0 (0)	1 (2.5)		
Chronic obstructive pulmonary disease	1 (2.5)	4 (10)[c]	0.19	0.02–1.80

[a] $P \leq 0.05$ Fisher's exact test; [b] $P \leq 0.01$ Fisher's exact test; [c] $P < 0.15$; [d] $P < 0.15$ not evaluable; (all patients were PCR-negative).

Table 3. Comparison of NIVEL criteria, ICHPPC-2 criteria, and GP's opinion.

	PPV(%)	NPV(%)	RR	95% CI
NIVEL criteria	52	NA	NA	NA
ICHPPC-2 criteria	54	85	2.4	0.3–18.3
GPs	76	75	6.8	1.4–33.3

NA = not applicable (NIVEL criteria are equivalent to inclusion criteria).

draw strong conclusions. The limited number of patients aged over 65 years can partly be explained by the high influenza vaccination coverage of almost 90% in this age group with a medical condition.[17] In this study, the most outstanding symptom correlated with influenza virus infection was cough which confirms the results of other studies.[18-20] More of importance however, is the period in which the influenza epidemic is seen, which stresses the importance of surveillance networks. During the yearly period of increased influenza activity the practitioner's intuition of which case was indeed influenza was accurate. Based on experience, the GPs are more likely to interpret better the weight of symptoms of the presenting patients. Results are possibly biased owing to two facts. First, the group of participating GPs was small and may not be representative. Second, the participating physicians used a trial protocol, which made them conscious of making the correct diagnosis and also may have led to a more stringent application of diagnostic labels than usual, resulting in a high overall predictive value of the GP's opinion.

The small number of patients included by the GPs is explained by two facts. First of all, the 1997/1998 winter season was a very mild influenza season in The Netherlands compared with other years. According to the NIVEL surveillance there was increased influenza activity for seven weeks (>5/10 000 inhabitants with ILI) and the epidemic threshold was not exceeded.[16] Another reason was the stringent inclusion criteria; to be able to perform sensitive confirmatory laboratory diagnosis we only included those patients who presented at the surgery within 48 hours of onset of symptoms. This group of patients presenting within 48 hours is also the target group for possible intervention with antiviral agents. Most patients in the Netherlands tend to consult their GP in a later stage of illness since the Netherlands General Practitioners (NHG) Standard advises to see patients when symptoms continue or worsen after five days of illness.[10]

Laboratory diagnosis of influenza virus by PCR was more sensitive compared with culture or rapid culture. The fact that other studies have mainly used culture, serology or antigen testing might have resulted in underestimation of influenza.[6,18-20] We have therefore chosen this method as a gold standard instead of the less sensitive isolation of influenza virus by culture. Although numerous studies have been performed to compare different laboratory diagnostic methods, including PCR, most of these studies do not take into account the problems of transport of the specimen from general practice to the laboratory.[22-25] Ideally, transportation of the samples should take place at 4°C. In practical terms, samples are sent by mail, overnight and at room temperature. The low recovery rate by culture in our study is very likely the result of viral inactivation during transport.

From our study we can conclude that using either the ICHPPC criteria or the sentinel criteria does not distinguish satisfactorily between influenza and other viruses/pathogens causing these symptoms. Intensification of the surveillance networks and notification of the results to GPs is one of the most powerful tools to diagnose influenza virus infection, since during the influenza season it seems to be less difficult to distinguish influenza from other respiratory virus infections. It would be interesting to look at a larger scale, because besides intensive virological sampling by a surveillance network, clinical scoring could be a useful diagnostic tool at hand for clinicians, especially when treating for influenza virus infection.

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