

Influenza and influenza-like illness in general practice: drawing lessons for surveillance from a pilot study in Paris, France

FABRICE CARRAT

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SUMMARY

Background. There are two types of influenza surveillance techniques: qualitative laboratory-based surveillance and quantitative medical practice-based surveillance. The former is of great importance in isolating new strains of the virus, which helps in the decision-making process concerning the composition of the vaccine, and the latter provides estimates of morbidity, mortality or economic impact as a result of infection from the influenza virus. Rapid methods such as immunofluorescence (IF) or immunocapture assays (ICA) are now available for diagnosis of influenza infections. However, little is known about the use of these methods for influenza surveillance purposes.

Aims. To evaluate the feasibility of a rapid influenza diagnosis in ambulatory conditions, and to investigate the therapeutical outcomes of patients suffering from influenza-like illness (ILI) in relation to the virological diagnoses.

Method. During the 1994–1995 influenza season, 130 patients presenting with ILI symptoms (<36 hours) to 33 general practitioners (GPs) were included in a prospective study. Two nasal swabs and one throat swab per patient were collected and sent to the laboratory within 12 hours. Information concerning therapeutical outcomes was recorded during examination. Specimens were analysed using the immunofluorescence (IF) method and antigen immunocapture assay (ICA).

Results. Sixteen influenza A (12%) and 19 influenza B (15%) infections were diagnosed. The overall rate of influenza positive specimens was 17% in the pre-epidemic period and 31% during the epidemic ($P = 0.1$). The rates of usable specimens for IF assay, nasal ICA and throat ICA were 46%, 100% and 99% respectively. The combination of these three collections ensured a highly sensitive influenza virological diagnosis. There were no differences in therapeutical outcomes between the influenza positive and negative cases. The GPs prescribed antibiotics in 60% of the cases for a mean duration of 7 days (± 1.2). The mean duration of sick leave was 3.4 days (± 1.6). Twelve patients (four influenza positive, eight influenza nega-

tive) had been vaccinated at the beginning of the winter. The practitioner's intuition concerning 'which patient should be tested for influenza virus' did not prove useful in improving the aptness of virological diagnoses in the field of influenza surveillance.

Conclusion. The only way to estimate the true impact of influenza is to carry out a systematic virological sampling based on a sensitive clinical definition and using sensitive laboratory methods.

Keywords: infectious diseases, influenza, general practice, diagnosis.

Introduction

INFLUENZA surveillance includes two types: qualitative laboratory-based surveillance and quantitative medical practice-based surveillance. The former is of great importance in isolating and analysing new strains of influenza viruses, and to provide help in the decision-making process leading to the composition of the vaccine.^{1,2} The latter has been developed in many countries to provide estimates of morbidity, mortality or economic impact owing to the influenza virus.^{3–10} Nevertheless, estimates of the disease burden associated with influenza are problematic because of the difficulty in distinguishing what is attributable to influenza from what is attributable to other respiratory pathogens. Moreover, when decision rules exist, which may lead to collecting specimens for a viral or a bacterial identification, they rarely rely on a well-defined sampling nor follow exact clinical criteria for the case-definition. Rapid methods such as immunofluorescence (IF) or immunocapture assays (ICA) are now available for diagnosis of influenza infections with high levels of sensitivity (above 80%) and specificity (close to 100%) in comparison with culture.^{11–13} However, little is known about the use of these methods in field conditions for influenza surveillance purposes.

During the 1994–1995 influenza season, a pilot study was designed in Paris to evaluate the feasibility of a rapid influenza diagnosis in ambulatory conditions (combining IF and antigen ICA methods) among patients suffering from influenza-like illness (ILI) seen in general practice. As a secondary objective, this study also provided the opportunity to investigate the therapeutical outcomes of ILI — which have rarely been considered in the influenza surveillance systems — and their possible relationships with the virological diagnoses.

Patients and Method

Thirty-three general practitioners (GPs) were enrolled during the winter and trained to collect nasal and throat swabs. Each eligible case was defined on the association of symptoms experienced for fewer than 36 hours (by detailed questioning of the patient) with one or more of the following criteria: ILI consultation, upper or lower respiratory tract infection, fever >38 °C without other infection being evident. Subjects younger than one-year-old were excluded. A signed consent form was required before

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inclusion. Two nasal and one throat swab specimens were collected from each patient. The first nasal swab was immediately smeared on two spots of a fluorescence microscopy slide; the second nasal and throat swab were placed in viral transport media (Virocult®). The laboratory received the specimens within 12 hours. There, the slide was fixed in cool acetone and the viral transport media were stored at -80°C until the assays had been performed. The slide was stained with fluorescent monoclonal antibodies for influenza A at one spot, and specific for influenza B at the other (IMAGEN®, Dako). The results were interpreted according to the smear cells' density. Viral transport media were used for influenza virus A or B detection using an antigen ICA specific to each virus. For the positive influenza A samples, two additional ICA were performed for an identification of the influenza A subtypes H1N1 or H3N2 whenever the quantity of the transport media was sufficient. The cut-off value was determined according to the negative controls optical density (OD) values (at least twice the negative controls OD mean) and to a lowest limit dilution of 0.5 units of hemagglutination (HA). An influenza positive case was defined on the basis of at least one positive assay.

During each patient examination, a questionnaire was completed. This included items concerning the general description of the patient, the use of vaccine, and actions taken due to illness (treatment, number of days of sick leave prescribed). Body temperature (rectal, oral or axillary $+0.5^{\circ}\text{C}$) was recorded twice; once at the onset of the disease (if possible) and then during examination. The GPs were also questioned regarding their opinion of the influenza aetiology of the case, prior to knowing the result of the virological diagnosis.

Between 13 March, 1995 and 30 April, 1995, an ILI epidemic was experienced in France as recorded by the GPs of the French computerized network of sentinel general practitioners (Sentinelle® system).¹⁴ In this database, each ILI case is defined by the association of a sudden fever of 39°C or more, respiratory symptoms, and myalgias. The decision to declare an epidemic is taken when the national weekly incidence rate exceeds the seasonal threshold for two successive weeks.¹⁵

One to two inclusions per GP were planned before the ILI epidemic, and up to four inclusions were requested during the epidemic period.

Data were analysed by chi square or Fisher's exact test (two-tailed) for qualitative variables, and by non-parametric one-way analysis of variance (Kruskal-Wallis) for quantitative variables.

Results

Between 5 February and 6 April, 130 patients were included in the study. The diagnosis was positive for 35 patients (27%) (Table 1), of whom six (17% of 36 patients) were isolated during the pre-epidemic period and 29 (31% of 94 patients) were isolated

Table 1. Positive virological diagnoses.

	IF nasal n (%)†	ICA nasal n (%)‡	ICA throat n (%)§	Total*
Influenza A	7 (12)	12 (9)	9 (7)	16 (12)
Influenza B	6 (10)	16 (12)	6 (5)	19 (15)
Total	13 (22)	28 (22)	15 (12)	35 (27)

*At least one positive result. † 60 slides, ‡ 130 nasal and § 129 throat swabs were analysed.

ed during the epidemic period ($P = 0.10$). Influenza A infection was diagnosed in 16 patients (four pre-epidemic and 12 epidemic). Influenza B infection was diagnosed in 19 patients (two pre-epidemic and 17 epidemic). Among the positive influenza A specimens, five were identified as H3N2 subtypes. Sixty (46%) of the slides were of suitable quality for the IF assay, whereas all the nasal swabs and nearly all the throat swabs collected for antigen ICA were usable. The rates of positive IF assays, nasal ICA and throat ICA were 22%, 22%, and 12% respectively.

There were no differences in age, sex, place of consultation or delay associated with influenza diagnoses. The mean age was 33 years, 59% of cases were women and 57% were examined at home (Table 2). The mean delay between the first symptoms and collection of specimens was 22 hours. The mean temperature at onset was 38.8°C and was 0.5°C lower during examination. Patients had used antipyretics before the consultation in 73% of cases. Twelve patients had been vaccinated at the beginning of the winter. The rate of influenza vaccination was not associated with influenza diagnoses (8/95 versus 3/16, versus 1/19, respectively).

GPs prescribed antibiotics in 56% of cases (62% penicillin and 25% macrolide) for a mean duration of 6.8 days, with no difference among the three groups (Table 3). A quarter of the patients continued their normal activities. For those on sick leave ($n = 59$), the mean duration was 3.4 days. Finally, the practitioners' answers concerning the influenza virus aetiology of the case were positive in 62% of the patients and not associated with laboratory diagnoses.

Discussion

This pilot study has highlighted the feasibility and the practicality

Table 2. Characteristics of the patients.*

	Negative (n = 95) n (%) m ± SD	Influenza A (n = 16) n (%) m ± SD	Influenza B (n = 19) n (%) m ± SD
Age (years)	32.4 ± 14	38.3 ± 18	32.6 ± 14
Sex			
male	40 (42)	5 (31)	8 (42)
female	55 (58)	11 (69)	11 (58)
Place of consultation			
surgery	40 (42)	8 (50)	8 (42)
home	55 (58)	8 (50)	11 (58)
Delay between first symptoms and collection of specimens (hrs)	21.8 ± 8	21.2 ± 7	20.6 ± 9
Temperature at onset ($\times 0.1^{\circ}\text{C}$)†‡	388 ± 6	387 ± 6	390 ± 7
Temperature during examination ($\times 0.1^{\circ}\text{C}$)‡	384 ± 7	384 ± 6	385 ± 8
Antipyretics before consultation	72 (76)	9 (56)	14 (74)
Vaccination	8 (8)	3 (19)	1 (5)

*No significant differences among the three groups. † Not taken for 13 patients ($n=84$ and 14 ; 19 respectively). ‡ Oral or rectal or axillary ($+0.5^{\circ}\text{C}$).

Table 3. Therapeutical outcome and viral hypothesis.*

	Negative (n = 95) n (%) m ± SD	Influenza A (n = 16) n (%) m ± SD	Influenza B (n = 19) n (%) m ± SD
Antipyretics	89 (94)	15 (94)	17 (89)
Antibiotics	56 (59)	8 (50)	9 (47)
Duration of the antibiotherapy (days)	7.2 ± 1.1	6.0 ± 1.4	6.8 ± 1.0
Duration of the sick leave prescribed (days)†	3.5 ± 1.7	3.0 ± 0.8	3.1 ± 1.2
Hypothesis about the influenza virus aetiology of the case			
no	38 (40)	6 (37)	5 (26)
yes	57 (60)	10 (63)	14 (74)

* No significant differences among the three groups. † For working-adults (n = 47, 4 and 8 respectively).

bility of a rapid influenza diagnosis performed by GPs in ambulatory conditions. In this article, we report on the use of IF and ICA methods for the diagnosis of influenza A and B infection. It has been shown that these methods are close to a 100% specificity but are not 100% sensitive in comparison with conventional culture on embryonated eggs or MDCK cells.¹¹⁻¹³ Furthermore, some difficulties are likely to arise during the collection of specimens, which may decrease the sensitivity of the virological diagnosis. This phenomena is well illustrated by the low rate of good quality slides for the IF assay, despite a medical training. We believe, however, that the combination of two different types of methods, the short delay between the onset of the disease (the collection) and the receipt of specimens at the laboratory, and the two locations of collection would all reduce the rate of false negative results and then guarantee high sensitivity. Note, for example, that two positive IF results (one influenza A and one influenza B) were obtained from patients negative in both nasal and throat ICAs, and that five patients had positive throat ICA but negative nasal ones.

For technical reasons, IF and ICA results were available within one day and within one week respectively. However, on request the GPs could have obtained these results within two hours; for example, in the case of severe or complicated ILI — although this situation did not arise during the study. A rapid influenza diagnosis is thus possible by the use of IF or ICA methods; the advantages compared to conventional methods such as culture or serology are two-fold. First, it provides useful information for the management of severe ILI, whether or not the diagnosis is positive. Secondly, it can be used to optimize prophylaxis prescription among contacts, particularly in the context of an influenza pandemic or among high-risk subjects.

The overall rate of influenza infection was 27% which agrees with the results from epidemiological studies based on virus isolates or serological surveys of ILI cases.^{5,7,10,16-18} As there was no difference in mean age between our sample and the 5230 cases of ILI reported by the sentinel GPs¹⁴ (mean age = 32 years; SD = 20.5), we could extrapolate the morbidity impact of the influenza outbreak in general practice in France at 1380 influenza infections per 100 000 inhabitants (adjusted for age and clinical definitions of ILI). This accounts for a total of 745 000 influenza consultations, of which about 50% were of type A/H3N2 and 50% of

type B.

The fact that influenza viruses can be isolated before an ILI epidemic is a common observation. In the Paris region, the first influenza virus was reported during the month of October 1994 by the World Health Organization national influenza centre. However, one surprising finding concerns the high value of the rate of positive influenza infections before the epidemic (17%). Five of the six pre-epidemic influenza viruses were isolated from 3 to 5 weeks before the onset of the ILI epidemic. This emphasizes the inability of laboratory-based surveillance systems, disconnected from medical practice-based surveillance systems, to handle the detection or the quantification of influenza epidemics. An interesting epidemiological result of our study concerned the therapeutical outcomes of ILI patients. Little information is available about the rate of antibiotics prescription or about the rate of sick leave for ILI cases in general practice. These parameters are, nevertheless, of key importance for economical studies about the disease burden of influenza in an active population. For example, we found that a quarter of the working patients voluntarily did not take sick leave during the disease, and that mean duration of that prescribed for the others was equal to 3.4 days (half the time expected¹⁹). Also, we have found no significant differences in therapeutical outcomes in relation to virological influenza diagnosis. This finding might be due, at least in part, to the small size of the sample (lack of statistical power). However, it may be a consequence of the special features of the 1994–1995 influenza season in France reflecting a moderate pathogenicity of the circulating influenza viruses: a late onset of the epidemic (the latest since the beginning of the ILI surveillance by sentinel GPs in 1984¹⁴), the moderate size of the epidemic compared with previous influenza seasons (e.g. winter 1989–1990), and the overlapping of two different types of influenza viruses during the epidemic period. Finally, the factors that influenced the therapeutical outcomes were associated with the practitioner's opinion regarding the influenza aetiology of the case, and were probably mostly correlated with the symptoms exhibited by the patients. GPs prescribed antibiotics in 49.4% of cases if they suspected an ILI due to influenza, versus 67.4% if they suspected an ILI attributable to another pathogen ($P = 0.045$). They prescribed antipyretics in 96.3% versus 87.8% ($P = 0.08$) of cases respectively.

In conclusion, the practitioner's intuition of 'which patient should be tested for influenza virus' could not be used as a strategy for improving the efficiency of virological diagnoses aimed at estimating the disease burden specifically associated with influenza. This is still the case in many of the combined laboratory and practice-based influenza surveillance systems. It is easy to show that, in this situation, the GP would select the most severe patients for an influenza diagnosis. This would lead to overestimating the individual effect of the disease (e.g. mean duration of sick leave per influenza case), and to underestimating the disease population impact (e.g. mean duration of sick leave for influenza per inhabitant). We believe that the only way to estimate the true impact of influenza (or equivalently, the impact of influenza vaccine) is to carry out a systematic virological sampling based on a sensitive clinical definition and using sensitive laboratory methods.

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