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ORIGINAL ARTICLE

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# Useful clinical findings and simple laboratory data for the diagnosis of seasonal influenza

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## Abstract

**Background:** When using rapid antigen test kits for the diagnosis of influenza, false-negative results may occur if done too soon after the onset of symptoms. The purpose of this study was done to determine clinical laboratory items other than rapid antigen testing are useful for diagnosing influenza.

**Methods:** The subjects were 915 patients who visited the outpatient clinic of hospital between April 2010 and March 2017 during the influenza epidemic seasons, from December to April, and had both fever of 37.0 degrees or more and cold symptoms.

**Results:** Of the 214 patients who met the inclusion criteria, 176 had influenza. Multivariate analysis extracted patient consultation within four days of onset, fever of 37 degrees or higher, posterior pharyngeal lymphoid follicles, CRP of 0.77 mg/dL or less, and a lymphocyte count of 900/μL less as independent variables.

**Conclusion:** In previous study, lymphoid follicles on the posterior pharyngeal wall and decreased lymphocyte count were reported as influenza-specific findings. Both were confirmed with high specificity in our study, indicating that both would be useful when patients with influenza-like symptoms were false-negative for the rapid antigen test.

KEYWORDS

influenza, lymphocyte count, posterior pharyngeal lymphoid follicles

## 1 | INTRODUCTION

Influenza virus infection is a major public health problem that affects 5%–15% of the global population annually,[1] with annual epidemics generally occurring from December to April. Epidemics of influenza virus infection lead to absenteeism from school, decreased workforce production, severe complications, and chronic illnesses, and create a burden on medical services. Because influenza spreads rapidly, early identification is important for optimal patient management and infection control. The classic influenza syndrome is sudden in onset and is characterized by fever, headache, cough, sore throat, myalgia, nasal congestion, weakness, and loss of appetite.[2-5]

Unfortunately, these symptoms are frequently seen in other respiratory infections caused by a variety of viral and nonviral pathogens. No single symptom is specific enough to be useful in differentiating influenza from these respiratory infections.[4]

The rapid influenza diagnostic test has become a useful tool for influenza diagnosis; however, false negatives may occur and delay the start of treatment, which increases the risk of serious disease.[6,7]

In a previous prospective study done in 2003 and 2004, pharyngeal influenza follicles were observed in patients with seasonal influenza A/H3N2.[8] Miyamoto et al reported that influenza follicles occur in both seasonal and novel influenza (A/H1N1pdm09).[9]

Of the common laboratory tests, nonspecific blood count analysis, C-reactive protein (CRP), and white blood cell count (WBC) are useful for differentiating a viral from a bacterial disease. Especially in the case of influenza, a decrease in the number of lymphocytes is common.[10]

Systematically combining symptoms and other information was reported to be a useful strategy.[11,12] The purpose of this study is to determine which clinical laboratory parameters other than rapid antigen test kit are useful for early diagnosis of the influenza in patients with influenza-like illness, even when the rapid antigen test kit was negative because of short disease duration.

## 2 | MATERIAL AND METHODS

### 2.1 | Study design

This is a retrospective case-control study of patients who visited hospital between April 2010 and March 2017 with influenza-like illness, with fever and other common symptoms as their chief complaints. We referred to the definition of influenza-like illness in the CDC and considered fever patients as patients who complained of fever with or without temperature measurement. In addition, sore throat, cough, and runny nose were also set as inclusion criteria.[13]

Medical examination was done by experienced general internal medicine doctors. Of the 8,886 patients who reported to our outpatient clinic, 915 had influenza-like illness during the epidemic seasons, between December and April of each year. After the exclusion of 701 patients, the data of 214 patients were available for inclusion. Of these, 176 were diagnosed as influenza group because of the positivity of rapid antigen test. Data on the following clinical items were collected: sex, age, body temperature, sore throat, cough, nasal discharge, headache, joint pain, digestive symptoms, pharyngeal redness, posterior pharyngeal lymphoid follicles, cervical lymphadenopathy, WBC, neutrophil count, lymphocyte count, and CRP. All rapid influenza virus antigen detection tests were performed in the hospital's laboratories. The kits were primed with a nasal swab sample. Patients with impaired consciousness or under age 15 were excluded. Fever was defined as an axillary measured body temperature of 37.0 degrees or higher. However, cases of 37.0 degrees or less due to the use of antipyretics at the time of medical examination were included. This study was approved by the Institutional Review Board (Reference number 17-8–05).

### 2.2 | Rapid influenza virus antigen detection test procedures

The immunochromatography-based RIDT Quick Navi-Flu (Otsuka Pharmaceutical Co., Ltd, Tokyo, Japan) was used in accordance with the manufacturer's instructions, which state that the time to a result is 5 min. Its sensitivities were 86.8% and 85.7%, and its specificities were 98.8% and 100% for influenza A and B, respectively.[14] The original antibody included in Quick Navi-Flu reacts with human-origin influenza virus (subtypes H1N1, H2N2, H3N2, and H5N1) and animal-origin influenza virus (subtypes H1 to H16) in vitro. Based on an immunochromatography method with these monoclonal antibodies, Quick Navi-Flu displays three lines: one for the detection of influenza A, one for influenza B, and a control.

### 2.3 | Statistical analysis

All statistical analyses were performed with EZR,[15] a modified version of R commander designed to add statistical functions frequently used in biostatistics. We regarded $P < .05$ as statistically significant. ORs and 95% confidence intervals (CIs) were used. Univariate analysis was done with all variables to determine differences between the influenza and not-influenza groups, and continuous variables were divided into two groups with cutoff values set. A multivariate logistic regression analysis was done using items with a $P$ value of $<.05$ as explanatory variables and the presence or absence of influenza as the objective variable. The discriminative ability of the multivariate model was evaluated by the ROC curve. The sensitivity, specificity, and likelihood ratio of the identified independent variables were calculated.

## 3 | RESULT

During the study period, 915 patients had influenza-like illness symptoms as their chief complaints. Of them, 701 were excluded, including 421 who took no influenza test, 93 with incomplete medical records, 18 with unknown body temperature, 93 with no WBC data, 56 with no CRP data, and 20 patients who visited the hospital from May to November.

This left the data of 214 patients available for study, 176 of whom were positive for influenza (Figure 1).

Table 1 shows the baseline characteristics of the patients with and without influenza. Sex, age, median symptomatic period, body temperature, joint pain, pharyngeal redness, posterior pharyngeal lymphoid follicles (Figure 2), WBC, lymphocyte count, and CRP were statistically different between the influenza and noninfluenza groups. The age of the influenza patients was significantly younger, the median of the symptomatic period was shorter, body temperature was higher, and they had more joint pain, pharyngeal redness, and posterior pharyngeal lymphoid follicles. The WBC, lymphocyte counts, and CRP in influenza patients were 6,100, 830, and 1.19, respectively. On the other hand, those in not-influenza patients were 7,550, 1,136, and 0.54, respectively. The WBC and lymphocyte counts of the influenza patients were lower than that of noninfluenza patients, and CRP was higher than that of the non-influenza patients. Table 2 shows the baseline characteristics of the

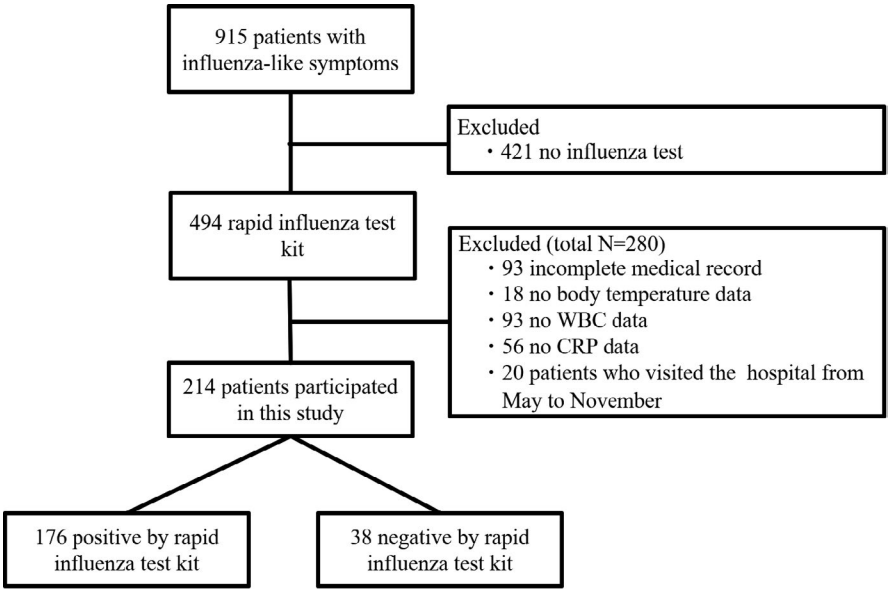


**FIGURE 1** Recruitment flow chart. The complete data of 214 of the 915 patients who reported to our outpatient clinic at Fukuoka University Hospital Between April 2010 and March 2017 were available for inclusion in this study. Of the 214 included, 176 had influenza

**TABLE 1** Clinical Characteristics

| | Influenza N = 176 | Noninfluenza N = 38 | *P* value |
|---|---|---|---|
| Sex; Male (%) | 67 (38.1) | 19 (50.0) | .02 |
| Age† (years) | 22 (20-36) | 27 (21-44) | .08 |
| Median of symptomatic period† | 3 (2-3) | 3 (2-4) | .30 |
| Body temperature (degrees)† | 38.0 (37.4-38.7) | 36.9 (36.6-38.0) | <.001 |
| Sore throat (%) | 94 (53.4) | 19 (50.0) | .7 |
| Cough (%) | 112 (63.6) | 21 (55.2) | .7 |
| Nasal discharge (%) | 85 (48.2) | 20 (52.6) | .7 |
| Headache (%) | 49 (27.8) | 10 (26.3) | 1.0 |
| Joint pain(%) | 46 (26.1) | 9 (23.6) | .8 |
| Digestive symptoms (%) | 11 (6.2) | 5 (13.1) | .2 |
| Pharyngeal redness (%) | 132 (75.0) | 27 (71.0) | .7 |
| Posterior pharyngeal lymphoid follicles (%) | 95 (53.9) | 11 (28.9) | <.001 |
| Cervical lymphadenopathy (%) | 29 (16.4) | 5 (13.1) | .8 |
| WBC(μ/mL)† | 6100 (4700-7025) | 7550 (6100-9725) | <.001 |
| Neutrophil count(μ/mL)† | 4439 (3355-5624) | 5658 (4503-7115) | <.001 |
| Lymphocyte count(μ/mL)† | 830 (614-1146) | 1136 (930-1644) | <.001 |
| CRP (mg/dL)† | 1.19 (0.48-2.30) | 0.54 (0.30-1.73) | .08 |

Abbreviations: CRP, C-reactive protein; WBC, White blood cell.
†Is the median

patients with influenza A and B. The WBC and CRP of the influenza patients were a little higher than that of noninfluenza patients. As shown in Table 3, multivariate logistic regression analysis extracted four factors: fever 37 degrees or over (Odds ratio (OR) = 4.63, 95% confidence interval (CI): 2.00-10.70, $P < .001$), posterior pharyngeal lymphoid follicle (OR = 2.71, CI; 1.17-6.28, $P < .001$), CRP 0.77 mg/dL or over (OR = 2.71, CI; 1.21-6.05, $P = .02$), and lymphocyte 900 μ/mL or less (OR = 3.42, CI; 1.38-8.46, $P = .01$), all of which were shown to have sufficient odds ratios to differentiate influenza patients from noninfluenza patients. The maximum area under the curve (AUC) of the receiver operating characteristic (ROC) was obtained for each of the three items extracted from the multivariate analysis (data not shown). The ROC curve of the scoring model shows an AUC of 0.804 (95%CI; 0.732-0.877). Table 4 shows the diagnostic characteristics of the four independent variables obtained by multivariate logistic regression analysis. In particular, fever 37.0 degrees or over,

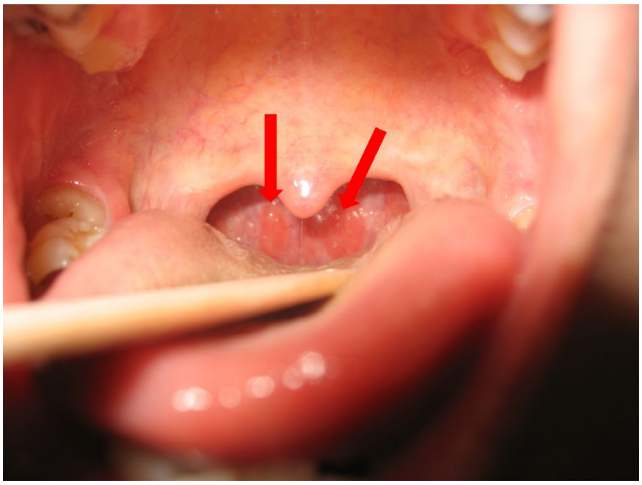

**FIGURE 2** A typical image of a definitive influenza follicles according to Miyamoto's[10] morphological classification of influenza follicles. The influenza follicles are aggregated in the part of the posterior pharyngeal wall (red arrows)

posterior pharyngeal lymphoid follicles, and lymphocyte 900 μ/mL or less had specificity, 85.8%, 54.0%, and 56.8%, respectively.

## 4 | DISCUSSION

In this retrospective study, we compared the results of rapid influenza virus antigen detection tests based on the reports of Caroline C et al.[16] The analysis of data in this study showed that fever 37.0 degrees or over, posterior pharyngeal lymphoid follicles, and lymphocyte 900 μ/mL or less were significant factors that can be used in the diagnosis of patients who had received false-negative rapid antigen test results for influenza.

Acute respiratory illness is a major cause of outpatient visits for patients of all ages. Most are viral diseases, but influenza viruses have high morbidity and mortality, making it important to distinguish them from other respiratory viral infections [17-19]. This analysis determined that there are clinical signs, symptoms, and laboratory data that would be of use in helping clinicians discriminate influenza infection from illness due to other respiratory viruses. Previous studies[2,20-22] reported that acute onset, fever, and cough were useful factors. Studies define cough definitions vary widely among the studies. In our study, cough was not significantly different, but fever above 37.0 degrees was extracted as significant in our multivariate analysis. It also has high specificity and is considered to be one of the most important symptoms of influenza.

It has been reported that posterior pharyngeal lymphoid follicles are significant diagnostic finding of influenza. As reported by Miyamoto and Watanabe[9], the sensitivity and specificity of influenza follicles observed 7.8 ± 5.3 h (range, 3-20 h; median, 5 h) after onset were 95.4% and 98.4%, respectively, for a seasonal influenza diagnosis. This characteristic finding for influenza patients can be observed in the early stage of influenza and thus is a useful factor for early diagnosis.

**TABLE 2** Influenza A or B Clinical Characteristics

| | Influenza A N = 137 | Influenza B N = 39 | *P* value |
|---|---|---|---|
| Sex; Male (%) | 52 (40.0) | 15 (38.5) | 1.00 |
| Age† (years) | 23 (20-36) | 21 (19-29) | .08 |
| Median of symptomatic period† | 2 (2-3) | 3 (2-3.5) | .30 |
| Body temperature (degrees)† | 38.0 (37.4-38.7) | 38.1 (37.5-38.5) | 1.00 |
| Sore throat (%) | 70 (51.0) | 24 (61.5) | .3 |
| Cough (%) | 90 (65.6) | 22 (56.4) | .3 |
| Nasal discharge (%) | 68 (49.6) | 17 (43.5) | .6 |
| Headache (%) | 36 (26.2) | 13 (33.3) | .4 |
| Joint pain(%) | 40 (29.1) | 6 (15.3) | .1 |
| Digestive symptoms (%) | 7 (5.1) | 4 (10.2) | .3 |
| Pharyngeal redness (%) | 101 (73.7) | 31 (79.4) | .5 |
| Posterior pharyngeal lymphoid follicles (%) | 75 (54.7) | 20 (51.2) | .7 |
| Cervical lymphadenopathy (%) | 23 (16.7) | 6 (15.3) | 1.0 |
| WBC(μ/mL) † | 6400 (5200-7500) | 4800 (4000-6150) | <.001 |
| Neutrophil count(μ/mL)† | 4722 (3727-5949) | 3524 (2834-4442) | <.001 |
| Lymphocyte count(μ/mL)† | 820 (608-1142) | 901 (662-1135) | .7 |
| CRP (mg/dL)† | 1.33 (0.60-2.50) | 0.70 (0.19-1.65) | <.001 |

Abbreviations: CRP, C-reactive protein; WBC, White blood cell.
†Is the median.

Blood tests showed a significant decrease in the lymphocyte counts of influenza patients. One study[23] reported that adult influenza patients who had no bacterial co-infection had normal or slightly reduced white blood cell counts and decreased lymphocyte counts. Lymphopenia can occur in either noninfectious or infectious diseases. Noninfectious diseases include autoimmune disorders such as systemic lupus erythematosus.[24] Because these diseases or conditions have their own clinical manifestations, we can exclude them. White blood cell count and lymphocyte count provide clues for the early detection of influenza infection; thus, regular blood tests such as white blood cell count and differentiation should be performed when managing patients with the symptoms of influenza-like illness. Furthermore, we compared and examined influenza A and B, but there was no significant difference in symptoms, and influenza A tended to be higher in WBC and CRP than influenza B, but it will be necessary to increase the number of cases with influenza B and examine in the future.

For retrospective study, there are limitations in this study. Due to the problem of false positives and false negatives of the rapid antigen test kit, there is a possibility that the person is not a true positive person or a true negative person. The prevalence is unknown,

**TABLE 3** Multivariable logistic regression analysis

| | OR (95%CI) | *P* value |
|---|---|---|
| 37.0 degrees or over | 4.63 (2.00-10.70) | <.001 |
| Posterior pharyngeal lymphoid follicles | 2.71 (1.17-6.28) | <.001 |
| CRP 0.77 mg/dL or over | 2.71 (1.21-6.05) | .02 |
| Lymphocyte 900 μ/mL or less | 3.42 (1.38-8.46) | .01 |

Abbreviations: CI, Confidence interval; OR: Odds ratio.

**TABLE 4** Diagnostic characteristics in diagnosis of seasonal influenza of each clinical item

| | Sensitivity (95%CI) | Specificity (95%CI) | Positive LR (95%CI) | Negative LR (95%CI) |
|---|---|---|---|---|
| 37 degrees or over | 0.858 (0.797-0.906) | 0.526 (0.358-0.690) | 1.81 (1.29-2.55) | 0.27 (0.17-0.43) |
| Posterior pharyngeal lymphoid follicles | 0.540 (0.463-0.615) | 0.711 (0.541-0.846) | 1.87 (1.11-3.13) | 0.65 (0.50-0.84) |
| CRP 0.77 mg/dL or over | 0.642 (0.566-0.713) | 0.605 (0.434-0.760) | 1.63 (1.08-2.45) | 0.59 (0.43-0.82) |
| Lymphocyte 900 μ/mL or less | 0.568 (0.492-0.642) | 0.789 (0.627-0.904) | 2.70 (1.44-5.06) | 0.55 (0.43-0.69) |

Abbreviations: CI, Confidence interval; LR, likelihood ratio.

and the search is difficult because the estimated number of true positives and true negatives changes every 10% of the prevalence. All the symptoms and sign were collected from the medical charts, which were recorded by the general medicine doctors, so some symptoms and signs may be missed. Control subjects are concentrated in young people. It is unknown how many times the influenza rapid antigen kit was used. There may not be performed until the blood collection in the clinic. The study was conducted during the epidemic of influenza, so it should be more cautious when applying these results during nonepidemic.

In this study, diagnosis and treatment was performed by specialists in general internal medicine. Future study including an increase in the number of cases will be necessary to eliminate possible bias in the decisions on the findings as made by the examining doctors.

We found fever 37.0 degrees or over, posterior pharyngeal lymphoid follicles, and lymphocyte 1000 μ/mL or less to be useful clinical findings that would enable clinicians to discriminate influenza from other influenza-like illnesses at the early stage of infection, before the accuracy of rapid detection kits can be guaranteed. This would be useful in clinical practice, as even patients false-negative by influenza kit could be diagnosed and treated at early stage of their illness. Further, it will be important to validate this model prospectively in diverse populations and settings and outside of the influenza season.

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## CONFLICT OF INTEREST

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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