Diagnosis of Influenza in the Community

Relationship of Clinical Diagnosis to Confirmed Virological, Serologic, or Molecular Detection of Influenza

Maria Zambon, PhD; John Hays, PhD; Alison Webster, MD; Robert Newman, MSc; Oliver Keene, MA, MSc

Background: Successful treatment of influenza depends on an accurate diagnosis of the illness and prompt intervention. However, there is a lack of data comparing clinical diagnosis vs laboratory diagnostic techniques.

Objective: To compare the clinical diagnosis of community cases of influenza with various laboratory diagnostic techniques including multiplex, reverse transcription polymerase chain reaction.

Methods: Clinical diagnosis, viral isolation, hemagglutinin inhibition serology, and multiplex, reverse transcription polymerase chain reaction were used to diagnose influenza in patients enrolled in international phase 3 studies designed to investigate the efficacy and safety of an antinfluenza drug (inhaled zanamivir). Patients clinically diagnosed with influenza were enrolled at centers across North America and Europe.

Results: A total of 791 (77%) of 1033 patients with laboratory results from all 3 methods were confirmed positive for influenza by 1 or more test results. For 692 patients (67%), the results of all 3 tests agreed. Total symptom scores at baseline showed a significant association toward greater severity of symptoms with an increasing number of positive test results ($P<.001$). An increasing number of positive test results also showed a significant correlation with a longer time to alleviation of symptoms of influenza in the placebo group ($P=.001$).

Conclusions: During a time when influenza was known to be circulating and clinical diagnostic criteria were applied, diagnosis of influenza in these trials was accurate in approximately 77% of adults on clinical grounds alone. This highlights the need for primary care physicians to be alerted to circulating influenza and to be aware that presentation with cough and fever provide the most predictive symptoms.

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PATIENTS, MATERIALS, AND METHODS

PATIENTS

Male or female patients, aged at least 12 years, initially seen within 48 hours of the onset of influenza-like illness were enrolled in the studies. Symptoms were defined as the presence of fever (temperature ≥37.8°C), unless the patient was ≥65 years, in which case, fever was defined as ≥0.2°C (temperature ≥37.2°C) and at least 2 of the following 4 symptoms: headache, myalgia, sore throat, and cough. All participants were required to give written informed consent.

All investigators had access to local and national influenza surveillance data and recruitment began only when influenza was confirmed to be circulating locally. The site was activated if more than 2 positive samples were reported in a 7-day period. The exclusion criteria were as described previously. Patients who had received influenza vaccine for the current season could be recruited in the study if confirmed as positive for influenza (IP) before the first study treatment by a rapid diagnostic test.

CLINICAL ASSESSMENTS

Global assessment of symptoms was made by the investigator at baseline on day 1 (first treatment visit, before study drug administration) using a 4-point scale (0 = none, 1 = mild, 2 = moderate, and 3 = severe). Patients recorded the presence and severity of the following 9 symptoms at baseline: headache, sore throat, feverishness, myalgia, cough, nasal symptoms, weakness, loss of appetite, and overall illness score, using the same 4-point scale. Patients also measured their baseline temperature using a tympanic thermometer before study treatment.

Patients continued to record the presence and severity of their symptoms and to take their temperature for 14 days or, if symptoms persisted, for 28 days. Alleviation of the main clinical symptoms was defined as follows: no fever (temperature <37.8°C), feverishness recorded as none, and a score of 0 or 1 (mild) for headache, myalgia, cough, and sore throat, all maintained for a further 24 hours. The incidence of influenza-related complications and associated antibiotic agent use was recorded throughout the studies.

LABORATORY ASSESSMENTS

Samples for diagnostic virologic testing, including nasal wash, nasal swabs, nasopharyngeal aspirates, throat swabs, and blood (serum) samples, were taken before treatment. Virus culture was performed using local isolation protocols, which involved inoculation of the clinical specimen onto either primary monkey kidney cells or Madin-Darby canine kidney cells, and was performed at a central laboratory for North American studies and in local laboratories in European countries. An aliquot of the same sample was stored at −70°C for subsequent PCR analysis. Influenza antibody titers at baseline and at day 28 were measured by hemagglutination inhibition serologic testing; assays were performed at the National Institute for Biological Standards and Control (Hertford, England) for both studies, according to standard protocols. Patients were considered to have shown seroconversion if the convalescent antibody titers were increased at least 4-fold compared with baseline.

Influenza diagnosis that included subtyping by multiplex RT-PCR was performed at the Public Health Laboratory Service, London, England, for both studies according to previously published methods. Stringent quality control procedures were used to ensure the accuracy, reproducibility, and quality of diagnosis.

STATISTICAL ANALYSIS

The intention-to-treat (ITT) population was defined as all randomized patients, whether or not the study drug was taken or the patient completed the study. Patients were considered to be IP if a positive result was obtained by culture or PCR assay, or if seroconversion was demonstrated by hemagglutination inhibition. All analyses included patients who had negative results on all 3 tests as positive on 0 tests.

Total symptom scores at baseline were calculated first as the sum of the 5 principal symptoms (ie, headache, sore throat, feverishness, myalgia, and cough) and second as the sum of all 9 symptoms (ie, headache, sore throat, feverishness, myalgia, cough, nasal symptoms, weakness, loss of appetite, and overall influenza assessment). Scores were expressed as a percentage of the maximum achievable score. Analysis of variance (ANOVA) was used to test for a statistically significant association between the total symptom scores and the number of positive test results. The presence of an association between the severity of each individual symptom on the original 4-point scale and the number of positive test results were assessed using the Mantel-Haenszel test. The ANOVA was also applied to test the relationship between baseline temperature and the number of positive test results.

Data on time to alleviation of symptoms of influenza, incidence of complications, and antibiotic agent use for complications was analyzed for the patients randomized to placebo. The presence of an association with the number of positive test results was assessed using the Mantel-Haenszel test.

Two international phase 3, double-blind, randomized, placebo-controlled, parallel-group studies (NAIA3002 and NAIB3002 described elsewhere) were designed to investigate the efficacy and safety of an inhaled anti-influenza drug in the treatment of symptomatic influenza type A and type B viral infections. The trials used a common study protocol and provided a unique opportunity to examine the relationship between clinically diagnosed influenza and laboratory confirmation ts-
RESULTS

A total of 1133 patients were enrolled in the European and North American studies. Baseline patient characteristics were similar in the ITT population and the population with results from culture, serologic testing, and PCR (Table 1). Data from all 3 methods were available for 1033 patients (91%). These patients, whether they were treated with placebo or oseltamivir, were included in the analysis of baseline diagnostic tests. A total of 791 evaluable patients (77%) clinically diagnosed with influenza were confirmed as IP with 1 or more tests. A total of 242 patients (23%) did not have a positive result in any laboratory diagnostic test and were categorized as influenza negative. Of the 100 patients with missing test results, 77 were missing for the day 28 serology only, 6 for serology and PCR, 12 for PCR only, and 5 for culture only.

Overall, in the 13 countries involved, 579 patients (56%) were positive by culture, 629 patients (61%) were positive by serology, and 730 patients (71%) were positive by PCR. Some variation in the positivity rates achieved by different diagnostic test results for patients initially seen within 48 hours of the onset of illness in the community when influenza was known to be circulating.

Concordance of results in patients with positive test results from all 3 diagnostic tests—serology (ie, nasal wash, nasal swabs, nasopharyngeal aspirates, throat swabs, and blood samples), virus culture (ie, inoculation of the clinical specimen onto either primary monkey kidney cells or Madin-Darby canine kidney cells), and multiplex reverse transcription polymerase chain reaction (RT-PCR).

For 692 (67%) of the 1033 patients with results from all 3 methods, the results of all 3 tests agreed. In 341 patients (33%), there was a discrepancy in the results of 1 test compared with the other 2 tests (Figure). Of the 791 IP patients, 450 patients (57%) were positive by all 3 methods. Where only 2 test results were positive (247 patients [31%]), serology and PCR gave the highest additional confirmation (126 patients [16%]), followed by culture and PCR (109 patients [14%]), and culture and serology (12 patients [2%]). A further 8 patients (1%) positive by culture, 45 patients (6%) positive by PCR, and 41 patients (5%) positive by serology were unconfirmed by any other test. However, in 29 (64%) of the cases with unconfirmed PCR results, the samples showed a serologic rise to the same subtype as detected by PCR, but the increase in antibody titers did not reach 4-fold, suggesting that these individuals had influenza infection but did not meet the scoring criteria for serologic positivity. In contrast, only 38 (16%) of 242 patients negative in all 3 methods showed any rise in antibody titers.

If culture and serology together are considered a gold standard for diagnosis of influenza, with a positive result on either test being considered evidence of influenza infection, then the sensitivity and specificity of diagnosis using RT-PCR was 92% and 84%, respectively.

Total symptom scores at baseline showed a significant association toward greater severity of symptoms with an increasing number of positive test results analyzed by ANOVA ($P<.001$; Table 3 and Table 4). Baseline temperature and the severity of cough, feverishness, nasal symptoms, loss of appetite (all $P<.001$), and weakness ($P=.01$) individually showed a statistically significant positive correlation with the number of positive test results. Sore throat showed a statistically significant positive association with results from culture, serology, and PCR ($P<.001$).
significant negative correlation with the number of positive test results \(P = .01\) suggesting that severity of this symptom was greater in patients who tested negative for influenza. Analysis of the 100 patients who did not have results from all 3 laboratory tests indicated that 55 had a positive result in 1 or more of their remaining tests. Comparison of those who had positive results on 2 tests with those who had negative test results indicates a rise in temperature and an increase or identical symptom score. Therefore, we conclude that there is no evidence of bias by the exclusion of patients with missing test results.

The influenza vaccine components for the study year were A/Wuhan/359/95 (H3N2), A/Bayern/7/95 (H1N1), and B/Beijing/184/93; whereas, the predominant circulating influenza strains were a mixture of A/Wuhan/359/95 and A/Sydney/5/97, a significant H3N2 drift variant from A/Wuhan/359/95. Of the 114 patients vaccinated for the season (with data from all 3 tests), confirmation of influenza was obtained in 92 patients (81%). The remaining 22 patients (19%) were not have results from all 3 laboratory tests indicated positive for influenza. Analysis of the 100 patients who did not have results from all 3 laboratory tests indicated positive for influenza. Analysis of the 100 patients who did not have results from all 3 laboratory tests indicated positive for influenza. Analysis of the 100 patients who did not have results from all 3 laboratory tests indicated positive for influenza. Analysis of the 100 patients who did not have results from all 3 laboratory tests indicated positive for influenza. Analysis of the 100 patients who did not have results from all 3 laboratory tests indicated positive for influenza. Analysis of the 100 patients who did not have results from all 3 laboratory tests indicated positive for influenza. Analysis of the 100 patients who did not have results from all 3 laboratory tests indicated positive for influenza. Analysis of the 100 patients who did not have results from all 3 laboratory tests indicated positive for influenza. Analysis of the 100 patients who did not have results from all 3 laboratory tests indicated positive for influenza. Analysis of the 100 patients who did not have results from all 3 laboratory tests indicated positive for influenza. Analysis of the 100 patients who did not have results from all 3 laboratory tests indicated positive for influenza. Analysis of the 100 patients who did not have results from all 3 laboratory tests indicated positive for influenza. Analysis of the 100 patients who did not have results from all 3 laboratory tests indicated positive for influenza. Analysis of the 100 patients who did not have results from all 3 laboratory tests indicated positive for influenza.

Of 783 patients who had missing baseline symptom scores, 765 had influenza type A (731 H3N2; 34 H1N1), and 18 had influenza type B. It was impossible to draw conclusions about the association of clinical scores or laboratory results with different subtypes because of the overwhelming predominance of H3N2 circulation. Concordance in the subtype of influenza identified by the different diagnostic tests was good. In 1 patient, PCR indicated influenza type B, whereas, serologic testing indicated type A. In 2 influenza A–positive patients, PCR indicated H3N2 and serologic testing indicated H1N1 infection. The reasons for the discrepancies of influenza subtype identified in 3 patients are unclear.

The natural course of influenza in these studies was followed by analyzing the time to alleviation of symptoms, incidence of complications (upper and lower respiratory tract, cardiovascular, and other complications of influenza as defined by the patient’s physician), and antibiotic agent use in the patients randomized to placebo (Table 6). There was no evidence of an association between the incidence of complications or antibiotic use for complications and the number of positive test results in patients who were IP and were

<table>
<thead>
<tr>
<th>Table 3. Baseline Individual Symptoms*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients With Results</strong>&lt;br&gt;From All 3 Tests</td>
</tr>
<tr>
<td>Severe headache</td>
</tr>
<tr>
<td>Severe sore throat</td>
</tr>
<tr>
<td>Severe feverishness</td>
</tr>
<tr>
<td>Severe myalgia</td>
</tr>
<tr>
<td>Severe cough</td>
</tr>
<tr>
<td>Severe nasal symptoms</td>
</tr>
<tr>
<td>Severe weakness</td>
</tr>
<tr>
<td>Severe loss of appetite</td>
</tr>
</tbody>
</table>

*Data are given as number (percentage) of patients.
†Seven patients had missing baseline symptom scores. Patients’ symptoms were scored on a 4-point scale (0 [none] to 3 [severe]).
‡P value uses all categories (none, mild, moderate, and severe).

<table>
<thead>
<tr>
<th>Table 4. Baseline Total Symptoms*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients With Results</strong>&lt;br&gt;From All 3 Tests</td>
</tr>
<tr>
<td>All patients, No.</td>
</tr>
<tr>
<td>Main symptom score§</td>
</tr>
<tr>
<td>Total symptom score§</td>
</tr>
<tr>
<td>Vaccinated patients, No.</td>
</tr>
<tr>
<td>Main symptom score§</td>
</tr>
<tr>
<td>Total symptom score§</td>
</tr>
<tr>
<td>Nonvaccinated patients, No.</td>
</tr>
<tr>
<td>Main symptom score§</td>
</tr>
<tr>
<td>Total symptom score§</td>
</tr>
</tbody>
</table>

*Data are given as mean ± SD unless otherwise indicated.
†Seven patients had missing baseline symptom scores. Patients’ symptoms were scored on a 4-point scale (0 [none] to 3 [severe]).
‡P value uses all categories (none, mild, moderate, and severe.).
§All symptoms are the following: headache, cough, sore throat, feverishness, myalgia, nasal symptoms, weakness, loss of appetite, and overall infection.
receiving placebo. However, a statistically significant association with longer time to alleviation of symptoms was found with an increasing number of positive test results (P= .001).

## Table 5. Test Results by Age Group*

<table>
<thead>
<tr>
<th>Age Group, y</th>
<th>Positive Result in All 3 Tests</th>
<th>Positive Result in Any 2 Tests</th>
<th>Positive Result in Any 1 Test</th>
<th>Negative Result in All 3 Tests</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-16</td>
<td>51 (58)</td>
<td>16 (18)</td>
<td>5 (6)</td>
<td>16 (18)</td>
<td>88</td>
</tr>
<tr>
<td>17-34</td>
<td>201 (43)</td>
<td>114 (25)</td>
<td>47 (10)</td>
<td>102 (22)</td>
<td>464</td>
</tr>
<tr>
<td>35-49</td>
<td>108 (38)</td>
<td>61 (21)</td>
<td>30 (11)</td>
<td>86 (30)</td>
<td>265</td>
</tr>
<tr>
<td>50-64</td>
<td>56 (39)</td>
<td>42 (29)</td>
<td>12 (8)</td>
<td>33 (23)</td>
<td>143</td>
</tr>
<tr>
<td>65+</td>
<td>34 (64)</td>
<td>14 (26)</td>
<td>0</td>
<td>5 (9)</td>
<td>53</td>
</tr>
<tr>
<td>All</td>
<td>450 (44)</td>
<td>247 (24)</td>
<td>94 (9)</td>
<td>242 (23)</td>
<td>1033</td>
</tr>
</tbody>
</table>

*Data are given as number (percentage) of patients.

## Table 6. Follow-up in Patients Receiving Placebo*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Positive Result in All 3 Tests</th>
<th>Positive Result in Any 2 Tests</th>
<th>Positive Result in Any 1 Test</th>
<th>Negative Result in All 3 Tests</th>
<th>Total</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients receiving placebo, No.</td>
<td>206</td>
<td>118</td>
<td>49</td>
<td>124</td>
<td>497</td>
<td>.82</td>
</tr>
<tr>
<td>Incidence of complications†</td>
<td>60 (29)</td>
<td>28 (24)</td>
<td>12 (24)</td>
<td>38 (31)</td>
<td>138 (28)</td>
<td>.48</td>
</tr>
<tr>
<td>Incidence of antibiotic use for complications†</td>
<td>37 (18)</td>
<td>16 (14)</td>
<td>6 (12)</td>
<td>27 (22)</td>
<td>86 (17)</td>
<td>.001</td>
</tr>
<tr>
<td>Time to alleviation of symptoms, median No. of days</td>
<td>7.0</td>
<td>6.0</td>
<td>5.0</td>
<td>5.0</td>
<td>6.0</td>
<td></td>
</tr>
</tbody>
</table>

*Data are given as number (percentages) of patients unless otherwise indicated.
†Includes upper and lower respiratory tract, cardiovascular, and other complications as defined by individual physicians.

The 2 international phase 3 clinical trials conducted with anti-influenza drugs provide a large set of clinical data allowing investigation of the relationship between clinical and laboratory diagnosis using a common clinical protocol. Of the patients who were clinically diagnosed with influenza, 77% were confirmed as IP by 1 or more laboratory test results. We can conclude that, during a time when influenza was known to be circulating and clinical diagnostic criteria were applied, diagnosis of influenza in these trials was accurate in approximately 77% of adults on clinical grounds alone. Other recent phase 2 and 3 trials for sialidase inhibitors, which did not include the use of RT-PCR for diagnosis, have shown that 59.6% of the 71% of patients initially seen with influenzalike illness had laboratory-confirmed influenza at a time when influenza was circulating.9-12 Enrollment criteria were similar for all studies, apart from documented fever which was not required in the Southern Hemisphere phase 3 trial8 or the European phase 2 trial.12 Our results show that baseline temperature and the severity of cough and fever showed a significant positive correlation with the number of positive test results and sore throat showed a significant negative correlation (Tables 3 through 5). This agrees with Monto et al13 who conclude that sore throat is a negative predictor of influenza and suggested that fever and cough are the best predictors of influenza.

In general, concordance between laboratory test results was reasonable, with 692 patients (67%) with results from all 3 tests having the same result in all tests. This may reflect the different sensitivity of the 3 diagnostic tests. As predicted from the findings of previous studies,3,11-18 PCR was the most sensitive diagnostic test; 92% of the patients who were IP were positive by PCR, compared with 80% of the patients who were IP and were positive by serologic testing, and 74% of the patients were IP and were positive by viral culture. Similar numbers of individuals were diagnosed with influenza infection using serologic criteria in both the placebo-treated and the drug-treated groups (data not shown), which supports the previous conclusion that zanamivir treatment does not interfere with the serologic response to influenza antigens.19

Neither culture nor serologic testing alone can be considered a gold standard for influenza diagnosis, mainly because each lacks sensitivity, and as shown here, is not universally in agreement, although both have been widely used as methods of diagnosing influenza in the past. While serology is a cheap and reliable diagnostic test,20 the requirement for paired samples limits the use of serology in the management of influenza and limits its usefulness as a diagnostic tool; most patients for whom diagnostic test results were incomplete were those failing to present for a day 28 serology test. Viral culture is well established and will continue to be a standard laboratory test in the diagnosis of influenza,21 although the drawbacks of length of procedure and requirement for infectious virus make it less useful for immediate patient management. In our studies culture testing missed 26% of IP cases identified by serologic testing or PCR. The sensitivity of RT-PCR against combined culture and serologic testing indicates that it should be considered the most sensitive test for diagnosis of influenza. In almost two thirds of cases where PCR was the only positive test result, there was also some serologic evidence of influ-
enza infection, suggesting that these were not false-
positive PCR results. In summary, we suggest that RT-
PCR could be considered the gold standard for diagnosis
of influenza, although further work is required to un-
derstand the relationship of RT-PCR detection of influ-
enza with presentation later in the course of an influenza-
lke illness, as these results refer to presentation in the
first 48 hours of an influenza-like illness. Several rapid
tests for the detection of influenza in a “near-patient set-
ting” have appeared in the marketplace in the last few
years.22,23 There are limited data on the sensitivity, spec-
cificity, and value for money of such tests and their place
in the management of influenza in the community
remains uncertain. However, the evaluation of such tests
will require knowledge of their predictive value against
other well-validated laboratory tests for influenza at a time
when influenza is circulating and consideration of per-
formance at different stages of the natural history of
influenza exists.

Variation between countries was observed in the pro-
portion of positive results obtained from the 3 labora-
tory tests. In particular, results from viral culture showed
marked variation between countries, possibly reflecting
the fact that this test was performed at several different
individual study centers and that local difficulties in trans-
portation of the samples might have occurred. Al-
though PCR and serology were performed centrally, varia-
tion in positive laboratory results between countries was
seen. Another contributing factor to the variation ob-
served is that few patients were recruited at some cen-
ters, such as in Holland and Spain; this was inevitable,
because outbreaks of influenza are unpredictable and can
vary in severity and prevalence.

Analysis of the total symptom scores and the labora-
tory diagnostic test results showed that the number of
positive test results correlated with the severity of symp-
toms at baseline. It is considered that increased severity
of illness is likely to be related to increased viral load,
although this was not formally evaluated in this study.
If this hypothesis is correct then the likelihood of de-
tecting virus by several different laboratory methods may
correlate with viral load and may be related to the na-
ture of the immune response, and, hence, with severity
and duration of illness.

The length of illness recorded in the patients re-
ceiving placebo was positively correlated with the num-
ber of positive diagnostic test results. The criteria used
to calculate the alleviation of the clinical symptoms of
influenza in these studies are a conservative measure of
recovery from influenza. The high incidence of compli-
cations and antibiotic use for complications in pa-
tients whose test results were negative for all 3 tests sug-
gests that while the influenza-like illness is shorter, the
risk of developing complications in these patients is simi-
lar to that for influenza.

The subtype of influenza may be related to the se-
verity of the illness. Influenza type A subtype H3N2 in-
fected is believed to be more severe than subtype H1N1
infection24 and influenza type A infection is generally
thought to be more severe than influenza type B in-
fec tion. Although in phase 2 and 3 clinical trials of zanamivir-
treated patients with both subtypes have similar
length and severity of disease, there were insufficient data
on H1N1 and influenza type B available to compare clini-
cal indicators of severity following infection with differ-
ent subtypes.

Of 114 patients in these trials with results from all
3 methods who had received the influenza vaccine in the
current season and were clinically diagnosed with influ-
enza, 92 were confirmed as IP with 1 or more labora-
tory diagnostic tests. In the year in which this study was
conducted (1997-1998), the vaccine strain for influenza type
A H3N2 was A/Wuhan/359/95, and circulating strains in-
cluded a mixture of A/Sydney/5/97, a drift variant of in-
fluenza type A H3N2, as well as A/Wuhan/359/95. The
effectiveness of influenza vaccines is 65% to 85% in years
where there is a good match between vaccine and circu-
lating strains.25 This study was not designed to investi-
gate the difference in influenza illness severity between
vaccinated and unvaccinated individuals, and it incor-
porates data from a few patients in a single influenza
season. Nevertheless, of the vaccinated patients in-
cluded in these trials, 80% had IP illness as severe as
that recorded in nonvaccinated patients; therefore, vac-
nination status should not automatically exclude the
diagnosis of influenza.

There is good correlation between clinical diagnosis
carried out by general practitioners in the community
and laboratory-confirmed diagnosis at times when
influenza is circulating. This highlights the need for pri-
mary care physicians to be alerted to circulating influ-
enza and to be aware that presentation with cough and
fever provide the most predictive symptoms. This will
allow accurate clinical diagnosis of influenza to be made
with confidence.

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Corresponding author: Maria Zambon, PhD, Influen-
za Unit, Enteric and Respiratory Virus Laboratory,
Central Public Health Laboratory, 61 Colindale Ave, Colin-
dale, London NW9 5HT, England (e-mail: mzambon
@phls.nhs.uk).

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