Peripheral blood lymphocyte-to-monocyte ratio as a screening marker for influenza infection

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ABSTRACT

Influenza outbreaks occur annually and account for significant morbidity and mortality. The overall burden of influenza infections, in the USA, for the 2017–2018 season, was an estimated 45 million cases, 810 000 hospitalizations and 61 000 deaths. Literature suggests that leukocyte count and differential, particularly lymphopenia and/or monocytosis, can provide diagnostic value for influenza infection. However, studies regarding these findings are limited in the adult population, particularly in the USA. The objective of this study was to determine if lymphocyte-to-monocyte ratio (L:M)<2 can be used as a screening marker for influenza infection. We performed a retrospective analysis of all patients who presented to University of Florida Health, Jacksonville, a university-affiliated tertiary care center in Jacksonville, Florida, between January 2017 and December 2018, with 'influenza-like' symptoms and who were subsequently admitted to the hospital. Patients were divided into two cohorts, based on whether they had laboratory-confirmed influenza versus another confirmed upper respiratory tract viral infection (influenza-like illness (ILI)). L:M was compared between the two groups and was found to be lower in the influenza group compared with the ILI group (p<0.0001). Results of this study demonstrate that a L:M<2 has significant diagnostic value in the acute phase of influenza and can be used for earlier detection and management of this disease, in order to improve clinical outcomes.

Significance of this study

What is already known about this subject?

- Influenza outbreaks occur annually and cause peaked winter epidemics with significant morbidity and mortality.
- Literature suggests that leukocyte count and differential, particularly lymphopenia and/or monocytosis, can provide diagnostic value for influenza infection.
- Small clinical studies have proposed using a ratio of lymphocytes-to-monocytes (L:M) below 2 as surrogate marker to distinguish influenza infection from influenza-like illness.

What are the new findings?

- An L:M ratio <2 can be used as a marker to diagnose influenza infection in the studied population.
- The optimal cut-off point for L:M ratio remains unknown, however when looking at different cut-off points in our study population, we found a ratio of <2 to be the spot where sensitivity and specificity seem to be most balanced.
- Decreasing the cut-off to <1.5 led to an increase in specificity, but compromised the sensitivity.
- Increasing the cut-off to <2.5 led to an increase in sensitivity, but compromised the specificity.
- The medians of L:M ratios were equal across inpatient units, intensive care unit units and patients whose admission resulted in death, therefore suggesting they cannot be implemented to predict disease severity.

How might these results change the focus of research or clinical practice?

- Physician recognition of the hematological effects of influenza virus may be particularly important for early detection, allowing prompt institution of respiratory precautions and administration of pharmacological therapy, consequently decreasing nosocomial spread of infection.

INTRODUCTION

Influenza infection encompasses a clinically defined respiratory illness caused by the orthomyxovirus family of single-stranded RNA viruses. Outbreaks occur annually and cause peaked winter epidemics with significant morbidity and mortality.[1] Patients present with non-specific upper respiratory and systemic symptoms including rhinorrhea, cough, fever, malaise, and myalgia, making it difficult to differentiate influenza from other respiratory viral pathogens.[2] Additionally, due to the wide range of presentations, attempts to develop clinical prediction tools for influenza infection have been highly unsuccessful.[2]

Diagnosis of influenza infection is currently completed through collection of nasopharyngeal specimens. Available tests are based on antigen detection and vary in both cost and complexity. The most sensitive and specific diagnostic test is reverse transcriptase-PCR (RT-PCR). Although recommended by the Infectious Disease Society

of America as the preferred confirmation test, it can take hours to result.[3] Conversely, point-of-care tests provide rapid results at the compromise of a decreased sensitivity of 50%–70%.[4]

Utilization of routinely available laboratory tests such as a complete blood count (CBC) with differential have become of particular interest as a screening marker for influenza infection. Prior studies have described the presence of both relative lymphopenia and relative monocytosis at the time of presentation in patients with influenza infection.[5] Small clinical studies have also proposed using a ratio of lymphocytes-to-monocytes (L:M) below 2 as surrogate marker to distinguish influenza infection from influenza-like illness (ILI).[3 6] Of these studies, a majority were conducted internationally, focused largely on a pediatric population, and had a relatively small sample size.[3 5 7] There is a paucity of data investigating L:M ratio <2 with influenza infection in the adult population, particularly in the USA. Furthermore, no prior studies have investigated utilization of L:M ratio as a clinical predictor for disease severity. The primary objective of this study was to examine if hematological markers, particularly an L:M ratio <2, can be used to identify patients with influenza A or influenza B infection.

MATERIALS AND METHODS

A retrospective analysis was completed on a study population of 323 patients admitted to University of Florida Health, Jacksonville, Florida, USA, between January 2017 and December 2018, for whom a respiratory pathogen panel was ordered and was positive for influenza infection (influenza A or influenza B) or ILI (rhinovirus/enterovirus, parainfluenza 1, parainfluenza 2, parainfluenza 3, parainfluenza 4, respiratory syncytial virus, human metapneumovirus, adenovirus). The patients were divided into two cohorts: the study population (n=151) included patients that tested positive for influenza A or influenza B, while the control group (n=172) comprised patients that tested positive for any ILI. Identification of influenza and ILI infection as reflected on the respiratory pathogen panel was completed via PCR of a nasopharyngeal swab.

Inclusion criteria were age ≥18 years, need for admission to the hospital and presence of a CBC and differential within 0–72 hours of respiratory pathogen panel testing. Exclusion criteria were age <18 years, history of autoimmune disease, malignancy, HIV, viral hepatitis, concurrent bacterial illness (bacteremia, bacterial pneumonia) and use of immunosuppressive medications including steroids.

Data were collected for subjects within each cohort including demographics (age, sex and ethnicity), respiratory pathogen panel results (influenza vs ILI), CBC and differential, presence of lymphopenia and/or monocytosis, L:M ratio, medical and social history, patient comorbidities, level of care required during admission (non-intensive vs intensive care) and clinical outcome (death vs no death). Reference ranges for clinical indicators were obtained via Epic electronic medical record. The two cohorts were compared to evaluate for differences among their hematological indices, particularly their L:M ratio.

Table 1 Patient characteristics

	Influenza (n=151)	Influenza-like (n=172)	P value
Age, median (Q1–Q3)			
Years	61 (51–72)	58 (47–72)	0.2140*
Sex, n (%)			
Female	88 (58.3)	91 (52.9)	0.3326
Race, n (%)			
African-American	96 (63.6)	94 (54.7)	0.1992†
Caucasian	53 (35.1)	72 (41.9)	
Other	2 (1.3)	6 (3.5)	
Social history, n (%)			
Alcohol	11 (7.3)	26 (15.1)	0.0275
Smoke	39 (25.8)	63 (36.6)	0.0372
Any drug use	13 (8.6)	23 (13.4)	0.1747
Medical history, n(%)			
Cardiovascular	13 (8.6)	25 (14.5)	0.0009
Respiratory	60 (39.7)	83 (48.3)	
Cardiovascular and respiratory	12 (8.0)	24 (14.0)	
None	66 (43.7)	40 (23.3)	

*Wilcoxon rank sum test.
†Fisher's exact test; χ^2 test.

STATISTICAL ANALYSIS

Descriptive statistics were counts and frequencies for categorical variables, and median (IQR) for continuous variables. Normality was checked with the Shapiro-Wilks test of normality. If a distribution of a continuous variable was found to be not normally distributed, Wilcoxon rank sum test was used for analysis. The χ^2 test, Fisher's exact test or the binomial proportions test was used to analyze categorical variables. The diagnostic accuracy of the predefined L:M ratio was measured by its sensitivity and specificity and the use of receiver operating characteristic curves. Simple logistic regression was used to describe the magnitude of association between L:M ratio and viral infection. All the analyses were performed using SAS for Windows V.9.4 (SAS, 2008).

RESULTS

The two groups did not differ statistically in age, sex, race or drug use distributions (table 1). The groups did differ in medical history. The influenza-like group had more instances of subjects with baseline cardiovascular disease (14.5%), chronic respiratory illness (48.3%) or both cardiac and respiratory medical history (14.0%), as compared with the influenza group (p=0.0009). Differences in clinical characteristics were statistically significant between the groups, with the exception of lymphopenia where we found no significant difference between groups (p=0.0792). The median L:M ratio however was lower in the influenza group (1.4, 0.9–1.9) than in the influenza-like group (2.4, 1.4–3.8) (p<0.0001) (table 2).

The sensitivity at the predefined threshold of <2 for L:M ratio was 76.2% (95% CI 69.4 to 83.0; p<0.0001) while the specificity was 60.5% (95% CI 53.2 to 67.8; p=0.0061). The positive predictive value (PPV) was 62.8% and the negative predictive value (NPV) was 74.3%. The overall

Table 2 Clinical characteristics

	Influenza (n=151)	Influenza-like (n=172)	P value
Visit details, n (%)			
CCU	2 (1.3)	18 (10.5)	<0.0001
MICU	26 (17.2)	47 (27.3)	
Inpatient	123 (81.5)	107 (62.2)	
Cell count, median (Q1–Q3)			
WBC	7.81 (5.9–9.9)	9.21 (6.5–11.9)	0.0016*
Platelets	198 500 (156 000–255 000)	221 000 (177 500–266 000)	0.0238*
Abs monocyte	0.70 (0.48–0.92)	0.56 (0.35–0.85)	0.0048*
Abs lymph	0.90 (0.63–1.39)	1.13 (0.73–1.92)	0.0019*
Monocytosis, n (%)			
Yes	109 (72.2)	104 (60.5)	0.0266
Lymphopenia, n (%)			
Yes	96 (63.6)	125 (72.7)	0.0792
L:M ratio, median (Q1–Q3)			
L:M	1.4 (0.9–1.9)	2.4 (1.4–3.8)	<0.0001*
Pathogen virus, n (%)			
Influenza A	1 (0.7)	--	
Influenza A (NAA)	99 (65.6)	--	
Influenza A (H3)	1 (0.7)	--	
Influenza B	50 (33.1)	--	
Adenovirus	--	6 (3.5)	
Human metapneumovirus	--	19 (11.1)	
Parainfluenza virus	--	1 (0.6)	
Parainfluenza virus type 1	--	7 (4.1)	
Parainfluenza virus type 3	--	11 (6.4)	
Parainfluenza virus type 4	--	6 (3.5)	
Respiratory syncytial virus	--	35 (20.4)	
Rhinovirus/Enterovirus	--	87 (50.6)	

*Wilcoxon rank sum test; χ^2 test.

performance of the predefined threshold had an area under the curve of 0.683 (95% CI 0.633 to 0.733; p<0.0001) (figure 1). In simple logistic regression analysis, the odds of being diagnosed with an influenza strain were five times greater for those with an L:M ratio <2 (OR 4.89; 95% CI 3.01 to 7.92; p<0.0001) (table 3). The viral subgroup analysis found significant differences in the proportions of L:M ratio <2 compared with >2 within each viral infection.

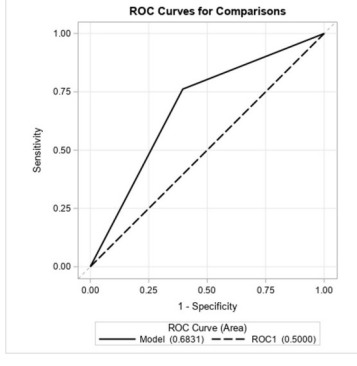

Figure 1 Lymphocyte-to-monocyte ratio <2 can correctly classify randomly drawn pairs of influenza/influenza like-subjects 68.3% of the time. ROC, receiver operating characteristic.

Influenza A and B had larger proportions of L:M ratio <2, 81.2% and 66.0%, respectively (table 4). The medians of L:M ratios were equal across inpatient units and intensive care units (ICUs) (online supplemental figure 1). The likelihood ratio for different thresholds of L:M ratios are displayed in table 5. At the predefined threshold of <2.0, a subject with influenza is 1.93 (1.53 to 2.32) times more likely to be classified as influenza positive compared with an influenza-like subject. After finding significant differences in the median platelet count between the influenza and influenza-like patients, the same method was applied to determine whether a patient's lymphocyte-to-platelet ratio (L:P) would have similar diagnostic accuracy. Analysis

Table 3 Overall diagnostic accuracy (n=323)

	Influenza	Influenza-like	OR (95% CI)	P value
<2 L:M	115	68	4.89 (3.01 to 7.92)	<0.0001
≥2 L:M	36	104	**AUC (95% CI)**	
	Sensitivity=76.2% (95% CI 69.4 to 83.0)	Specificity=60.5% (95% CI 53.2 to 67.8)	0.6831 (0.633 to 0.733)	<0.0001
	P<0.0001*	P=0.0061*		

*Simple logistic regression; binomial proportions test.
AUC, area under the curve; L:M, lymphocyte-to-monocyte ratio.

revealed that L:P ratio was not a significant predictor of influenza infection (p=0.2487).

DISCUSSION

Seasonal influenza infection has a significant disease burden worldwide and contributes to a large proportion of hospitalizations and deaths annually. According to WHO, influenza infection is estimated to cause 290 000–650 000 respiratory deaths alone.[4] It is primarily transmitted between individuals through close contact with large-particle respiratory droplets, however transmission can also occur through contact with droplet contaminated surfaces. Utilization of non-specific abnormalities from a patient's CBC can prompt physicians to consider influenza infection in a patient presenting with systemic and upper respiratory symptoms, allowing for earlier detection and management.

Prior studies have recognized the presence of lymphopenia and monocytosis in seasonal influenza infection, however limited evaluation has been done in the USA, particularly in the adult population. Our study demonstrates the presence of hematological abnormalities in adult patients with influenza infection which warrant further investigation when present in the appropriate clinical context. An L:M ratio <2 in the acute phase of infection may be a simple way to identify influenza infection and differentiate it from ILI. Influenza-infected individuals are considered contagious from the day prior to symptom onset until 5–10 days thereafter. Physician recognition of the hematological effects of influenza virus may be particularly important for early detection, allowing prompt institution of respiratory precautions and administration of pharmacological therapy, consequently decreasing nosocomial spread of infection.[8]

In our study population, the ILI population had more instances of ICU admissions compared with the influenza cohort. While this can in part be due to a larger sample size of the ILI population, it is more likely explained by differences in the underlying medical conditions between the two groups. Compared with the study population, subjects within the ILI group had increased baseline cardiovascular, respiratory or both cardiovascular and respiratory comorbidities. The medians of L:M ratios were equal across inpatient units, ICU units and patients whose admission resulted in death, therefore suggesting they cannot be implemented to predict disease severity.

Our results demonstrated that an L:M ratio <2 can be used as a marker to diagnose influenza infection in the studied population. However, because the prevalence of influenza and ILI varies within different regions and patient populations, generalizability cannot be determined. Establishing similar accuracy across multiple settings to determine if the sensitivity and specificity of an L:M<2 is consistent with our findings requires further investigation. Additionally, evaluation of disease prevalence in various regions is required to determine if the PPV and NPV of the screening test are similar. Our research focused exclusively on individuals in the inpatient setting, thereby limiting utilization in the outpatient context. Moreover, multiple patient populations were excluded, including those with malignancy, HIV, hepatitis and other immunodeficiencies. Leukocyte counts of individuals within these patient populations have not yet been evaluated and warrant further investigation.

A prior study completed a longitudinal analysis of leukocyte differentials in the peripheral blood of patients with acute respiratory viral illnesses.[9] Findings of this research suggested that the presence of lymphopenia and relative monocytosis closely mirror symptom development in time. Leukocyte differentials from our study were obtained within 0–72 hours on completion of a respiratory pathogen panel via nasopharngeal swab analysis. These results do not provide insight into the temporal development of hematological abnormalities and time-dependent utility of an L:M<2 in diagnosis of influenza infection.

Lastly, there are currently no clinical prediction tools to distinguish influenza from other respiratory viral pathogens. The results of our investigation suggest that L:M ratio could potentially be implemented to develop clinical prediction scales in the future. The optimal cut-off point for L:M ratio remains unknown, however when looking at different cut-off points in our study population, we found a ratio of <2 to be the spot where sensitivity and specificity seem to be most balanced. Decreasing the cut-off to <1.5 led to an increase in specificity, but compromised the sensitivity. Increasing the cut-off to <2.5 led to an increase in sensitivity, but compromised the specificity (table 5).

In conclusion, our study demonstrated that L:M ratio <2 has significant diagnostic value in the acute phase of influenza infection and can potentially be used for earlier

Table 4 Viral subgroup analysis

	<2 L:M	≥2 L:M	
	% (95% CI)	% (95% CI)	P value
Influenza A	81.2 (73.6 to 88.8)	18.8 (11.2 to 26.4)	<0.0001
Influenza B	66.0 (52.9 to 79.1)	34.0 (20.9 to 47.1)	0.0237
Influenza-like	39.5 (32.2 to 46.8)	60.5 (53.2 to 67.8)	0.0061

Binomial proportions test.
L:M, lymphocyte-to-monocyte ratio.

Table 5 Diagnostic accuracy at different thresholds

	+LR	−LR	Sensitivity	Specificity	OR
<1.5 L:M	2.02 (1.42 to 2.62)	0.65 (0.53 to 0.77)	51.7% (43.7 to 59.6)	74.4% (67.9 to 80.9)	3.11 (1.95 to 4.96)
<2.0 L:M	1.93 (1.53 to 2.32)	0.39 (0.27 to 0.52)	76.2% (69.4 to 83.0)	60.5% (53.2 to 67.8)	4.89 (3.01 to 7.92)
<2.5 L:M	1.66 (1.39 to 1.94)	0.32 (0.19 to 0.45)	84.1% (78.3 to 89.9)	49.4% (42.0 to 56.9)	5.17 (3.05 to 8.77)

Estimate (95% CI).
L:M, lymphocyte-to-monocyte ratio; LR, logistic regression.

detection and management of this disease in the inpatient adult population.

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