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IP Indian Journal of Immunology and Respiratory Medicine

Journal homepage: https://www.ijirm.org/

Original Research Article

Uncovering the diagnostic value of white blood cell profiles in H1N1 screening

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ARTICLE INFO

Article history:
Received 20-11-2023
Accepted 13-12-2023
Available online 27-01-2024

Keywords:
H1N1
White blood cell
Monocytes
RT PCR
Influenza
Monocytosis

ABSTRACT

Background: This research sought to evaluate the effectiveness of White Blood Cell (WBC) profiles as a screening method for promptly identifying H1N1 infection in individuals exhibiting symptoms of Influenza-like Illness (ILI) and undergoing throat swab testing for H1N1 through the RT-PCR technique.
Materials and Methods: Seventy patients from a tertiary care hospital were included based on specific inclusion and exclusion criteria. Throat swab tests were conducted using RT-PCR, resulting in 35 positive and 35 negative cases for influenza A H1N1. Complete Blood Count (CBC) profiles, including total WBC counts, differential count, neutrophil to lymphocyte ratios, lymphocyte to monocyte ratios, and absolute monocyte count (AMC), were obtained for all subjects.
Results: Analysis of the obtained data revealed that increased symptoms, higher monocyte counts, and an Absolute Monocyte Count (AMC) exceeding 800 cells/mm^3 were indicative of a higher likelihood of H1N1 positivity.
Conclusion: The study suggests that utilizing WBC profiles, particularly the neutrophil to lymphocyte ratios, lymphocyte to monocyte ratios, and AMC, can serve as a valuable screening measure for the early detection of H1N1 infection. This approach may be especially beneficial in peripheral healthcare settings where the standard RT-PCR diagnostic method is time-consuming, enabling timely and appropriate intervention based on CBC results.

1. Introduction

Pandemic influenza is a global outbreak of new human influenza and developing rapidly which seems rare and unpredictable. Worldwide, the yearly attack rate for influenza is estimated to be 5–10% in adults and 20–30% in children.[1]

Only viruses of the H1N1, H2N2, and H3N2 subtypes have been persistently circulating in humans. Since their appearance in 1997 and 2013, respectively, avian influenza viruses of the H5N1 and H7N9 subtypes have infected hundreds of people, but have not successfully spread among humans.[2] Additionally, reports of isolated human infections with viruses of many additional subtypes, including H9N2, H6N1, H7N7, H10N8, H7N2, and H7N3, have been made.[2]

"Influenza-like illness (ILI) is defined as fever (temperature of 100ºF [37.8ºC] or greater) with cough or sore throat in the absence of a known cause other than influenza".[3]

A diagnosed instance of pandemic H1N1 influenza A is characterized as an individual displaying symptoms of Influenza-like Illness (ILI) whose H1N1 influenza a virus presence is confirmed through laboratory testing via real-time reverse transcriptase polymerase chain reaction (RT-PCR) or culture.

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https://doi.org/10.18231/j.ijirm.2023.028

Infection with the influenza A (H1N1) virus was linked to considerable morbidity, mostly in children and young people.[4] The majority of patients had mild to moderate, simple diseases that self-limited. But several patients experienced serious disease, and some of them passed away. The most frequently documented complications in hospitalized adults included primary pneumonia, secondary bacterial pneumonia, exacerbations of chronic obstructive pulmonary diseases, the necessity for intensive care unit admission, including mechanical ventilation, acute respiratory distress syndrome, septic shock, nosocomial infections, and acute cardiac events.[5] The impact of the H1N1 virus seemed to be predominantly focused on the lower respiratory system, leading to diffuse alveolar damage. This was clinically evident through severe acute respiratory distress syndrome characterized by refractory hypoxemia, contributing to heightened morbidity and mortality among hospitalized patients.[5]

Some studies states that hematological abnormalities such as white blood cell (WBC) profile, which includes the neutrophil/lymphocyte ratio, the lymphocyte/monocyte ratio, and the monocyte counts used as screening tool in H1N1 virus.[6–8]

Limited studies are available worldwide where the role of WBC count and its differential count has been correlated with H1N1 diagnosis (as a screening tool). Standard modality of diagnosis of H1N1 by RT- PCR test is time consuming (approximately 3 days) especially in peripheral setup where this test as a screening tool may help to act appropriately in time. H1N1 infection is rapidly progressing & contagious illness. Thus high clinical suspicion, aided with strongly supportive WBC profile may help in early diagnosis, isolation & definitive care at an earlier stage. As the global spread of infectious diseases continues to be a significant public health concern, the need for timely and accurate screening methods becomes increasingly important.

The objective of this study was to assess the effectiveness of White Blood Cell (WBC) profiles as a screening tool for promptly identifying H1N1 infection in patients with Influenza-like Illness (ILI) who underwent throat swab testing for H1N1 using the RT-PCR method. This investigation was conducted in a tertiary care hospital setting.

2. Materials and Methods

After obtaining the clearance from institutional ethical committee, this cross-sectional study carried out in the department of respiratory disease at Lilavati Hospital and Research Centre (LHRC), Mumbai during March 2015 to March 2017 and the patients presenting with influenza like symptoms such as cough, fever, myalgia, breathlessness, head ache, chills, running nose were enrolled. Informed consent were obtained from all the patients included in the study. Patients younger than 18 years old and who are receiving chemotherapy and radiation treatment for hematological malignancies such as leukemia, lymphoma, and myeloma were excluded from the study. Patients are selected randomly whose nasopharyngeal swabs positive for H1N1virus by RT-PCR technique were classed as H1N1 positive group and negative nasopharyngeal swab report classed as HIN1 negative group. The study group comprised a total of seventy patients, with thirty-five testing positive for influenza H1N1 and an equal number of thirty-five testing negative for influenza H1N1, encompassing individuals of both genders. Within 72 hours of sickness, laboratory data were gathered for each patient in two groups, which included Complete Blood Count (CBC) profile and neutrophil to lymphocyte ratio, lymphocyte to monocyte ratio and absolute monocyte count were calculated.

2.1. Statistical analysis

Qualitative data was represented in form of frequency and percentage. Quantitative data was represented using Mean ± SD. Analysis of Quantitative data between H1N1 status (Positive/Negative) was done by Mann-Whitney Test. Binary Logistic Regression was used to assess predictors of H1N1 status (Positive/Negative) with 'H1N1 status' as dependent variable and a set of independent (Predictor) variables.

3. Results and Discussion

A total of 70 patients who presented with influenza-like illness (ILI) were enrolled in the study; 35 of these patients tested positive for the H1N1 virus and 35 tested negative based on the results of a nasopharyngeal throat swab (by RT PCR). Among these 82 % of H1N1 positive patients belonging to the age group 18-57years and 65% of H1N1 negative belonged to above 58 years of age. H1N1 positive is more common in young and middle-aged people are consistent with earlier investigations.[9,10] This can be explained by the fact that older people, who had previously been exposed to influenza viruses with antigenically similar genetic makeup, were more protected against the H1N1 virus than middle-aged and younger persons.[11,12]

Table 1 provides the status of CBC profile of the study population. Out of 70 patients with ILI, 82.85% (58/70) patients had normal WBC level, leucocytosis was found only in 12.85% (09/70) subjects i.e 5.71% (02/35) in H1N1 positive and 20% (07/35) in H1N1 negative group and 4.28%patients had leucopenia. The association of WBC level and H1N1 status was found to be statistically not significant. Our findings are consistent with the study conducted by Dong Hyuk Shin et al.[13] Thus, an influenza like illness with comparatively a normal range of leucocyte counts may raise a suspicion for H1N1 infection only but cannot be taken as a marker of the same. All the more,

Table 1: Complete blood count profile of H1N1 positive and negative cases of the study population

Variables	H1N1	Mean ± SD	p-value
WBC count (4000-11000 CU mm)	Positive	7390.68± 2354.84	0.194
	Negative	8914.36± 4394.94	
N/L <2	Positive	5.83 ± 4.97	0.272
	Negative	6.91 ± 5.33	
L/M <2	Positive	3.61 ± 4.51	0.643
	Negative	4.19 ± 4.91	

[WBC-White blood cell, N/L- Neutrophil Lymphocyte ratio, L/M- Lymphocyte Monocyte ratio]

Table 2: Binary logistic regression with 'H1N1 status' as dependent variable and a set of independent (Predictor) variables.

Variables	B	S.E.	Wald	p-value	Exp(B)
No. of Symptoms	0.748	0.287	6.776	0.009	2.113
N/L <=2	0.808	0.773	1.094	0.296	2.244
L/M <=2	-1.244	0.707	3.097	0.078	0.288
Monocyte count	-1.726	0.826	4.366	0.037*	0.178
AMC > 800 cumm	1.724	0.865	3.969	0.046*	5.606
Lymphopenia status	0.023	0.753	0.001	0.976	1.023
Constant	-3.407	1.216	7.853	0.005	0.033

[N/L- Neutrophil Lymphocyte ratio, L/M- Lymphocyte Monocyte ratio, AMC- Absolute monocyte count, p value* - <0.05 considered as statiscally significant]

leucopenia can be a suggestive tool but cannot be taken as a surrogate marker for H1N1 infection. Leukocytosis when present, typically indicates supper added bacterial infection, which always stays a possibility.

Table 2 shows the variables such as number of symptoms of H1N1, N/L <=2, L/M <=2, Monocyte 800, AMC > 800cumm and Lymphopenia status. No. of Symptoms, more the monocyte count and AMC > 800 cumm are statistically significant predictor of H1N1 status. Presence of more symptoms, more the monocytes and AMC more than 800 cumm shows the more probability of H1N1 positive status.

4. Discussion

In a research conducted by Merakoulius G et al[8] in Greece in 2009, the combination of monocytosis and lymphopenia, or a reduced ratio of lymphocytes to monocytes (<2), along with normal or low total white blood cell count, was suggested as a potential screening tool for influenza infection, as an alternative to rapid tests. A prospective study conducted by Voudoukis E et al[14] on H1N1 patients at Greece in 2009 found that monocytosis was seen in 55.6% (45/81) of H1N1 positive patients as compared to 27.4% in H1N1 negative group (23/84). A study conducted by in a study conducted by Coskun-o et al[15] in Turkey in 2009, it was noted that elevated white blood cell values were observed in 30.4% of Influenza-like Illness (ILI) cases, but only in 16.1% of H1N1 patients. Specifically, among all white blood cell types, the neutrophil count exceeded reference limits in 43.5% of ILI cases and 32.3% of H1N1 cases. Monocytes were increased in 16% of ILI and 30% of H1N1 cases and lymphopenia was seen in 27% of H1N 1 cases. They observed that the possibility of monocytosis and lymphopenia could be regarded as highly probable in H1N1 cases. Moreover neutrophil count was significantly high in ILI patients; this parameter was also increased in non-viral infections and it might cause misleading in the evaluation of H1N1 patients.[15]

In the study conducted by Cunha BA and colleagues,[16] complete blood counts were conducted on 25 adults and 16 children who tested positive for influenza A (using a rapid antigen test) with probable H1N1. Relative lymphopenia (comprising 21% of white blood cells) was observed in 92% of adults, while thrombocytopenia was present in 28% of adults with probable H1N1 infection. No leukopenia was detected in any of the 25 adults. Consequently, among adults testing positive for influenza A through rapid testing, relative lymphopenia appears to serve as an indicator for identifying those likely to have H1N1, thereby warranting specific RT-PCR testing.[16]

In a research undertaken by Indavarapu A and colleagues[17] in Hyderabad in 2010, it was discovered that a Neutrophil-to-Lymphocyte ratio (N/L) of less than 2, coupled with a reduction in White Blood Cell (WBC) count, could serve as a screening tool for patients exhibiting symptoms resembling influenza. This approach, used while awaiting throat swab culture reports for confirmation, demonstrated a sensitivity of 92.98% and specificity of 96.36%. A study conducted by Vijapura A et al[9] on H1N1(only category-C) patients at Gujarat in 2015 observed that Polymorphonuclear to lymphocyte ratio (P/L<2) was in 38.23% patients and lymphocyte-to-monocyte ratio (L/M <2) was observed in 2.94% patients. Study showed that neutrophil–lymphocyte ratio and lymphocyte–monocyte ratio cannot be used as a

criteria of exclusion of H1N1 infection because of higher prevalence of secondary bacterial infection.

Our observations were in concordance with most of the literature available where H1N1 patients had significant monocytosis but not with N/L<2, L/M<2. The usual finding of H1N1 infection on differential leucocyte count i.e N/L <2 and L/M <2 was not observed in our subpopulation which may be due to suspected superadded bacterial infection which lead to relative neutrophilia and lesser monocytosis. Also, we had a smaller sample size with a mix of stable and sick patient profiles. However, lymphopenia was a finding noted in all the patients with influenza like illness irrespective of H1N1 positive or H1N1 negative status. Thus, our findings were in accordance to Vijapura A et al[8] were also super added bacterial infections altered the usual immune response against H1N1 infection seen by others as N/L<2, L/M<2. However we did not analyze objective parameters for supporting a superadded bacterial infection in patients like procalcitonin, C-reactive protein, blood and body fluid culture. It is possible that P/L ratio and L/M ratio <2 may be present in uncomplicated H1N1 infection as seen in other studies. But, it cannot be used as criteria of exclusion of H1N1 infection because of high prevalence of secondary bacterial infection. Polymorphonuclear predominance does not rule out H1N1 infection.

5. Conclusion

Considering H1N1 influenza as a potential differential diagnosis is crucial when patients exhibit Influenza-like Illness (ILI) along with a variety of symptoms. Monocytosis (Absolute Monocyte Count >800 cells/mm^3) during the initial presentation could serve as a valuable marker for the early diagnosis and initial management of suspected H1N1 cases. This is particularly relevant when awaiting throat swab reports, especially in situations where there is a potential for secondary bacterial infection at the time of presentation.

We propose that this observation be further explored in more extensive study populations, encompassing various age groups, including children, adults, and pregnant women. Investigating whether any Complete Blood Count (CBC) parameters can function as a time-saving and cost-effective screening test for H1N1 virus infection is essential. Implementing such a tool could facilitate early antiviral treatment and contribute to a reduction in the incidence of complicated cases. This approach would be especially beneficial in regions where laboratory confirmation is constrained due to financial limitations or overwhelming demand.

6. Conflict of Interest

None.

7. Source of Funding

None.

References

1. World Health Organization. Vaccine standardization influenza; 2023. [Updated on January 16, 2023]. Available from: https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/vaccine-standardization/influenza.
2. Koopmans M, Wilbrink B, Conyn M, Natrop G, Van Der Nat H, Vennema H, et al. Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. *Lancet*. 2004;363(9409):587–93.
3. Shah SC, Rumoro DP, Hallock MM, Trenholme GM, Gibbs GS, Silva JC, et al. Clinical predictors for laboratory-confirmed influenza infections: exploring case definitions for influenza-like illness. *Infect Control Hosp Epidemiol*. 2015;36(3):241–8.
4. O'Riordan S, Barton M, Yau Y, Read SE, Allen U, Tran D, et al. Risk factors and outcomes among children admitted to hospital with pandemic H1N1 influenza. *CMAJ*. 2010;182(1):39–44.
5. Viasus D, Revuelta JO, Martínez-Montauti J, Carratalà J. Influenza A(H1N1)pdm09-related pneumonia and other complications. *Enferm Infecc Microbiol Clin*. 2012;4(Suppl 4):43–8.
6. Fouchier RA, Munster V, Wallensten A, Bestebroer TM, Herfst S, Smith D, et al. Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. *J Virol*. 2005;79(5):2814–22.
7. Kumar S, Henrickson KJ. Update on influenza diagnostics: lessons from the novel H1N1 influenza A pandemic. *Clin Microbiol Rev*. 2012;25(2):344–61.
8. Merekoulias G, Alexopoulos EC, Belezos T, Panagiotopoulou E, Jelastopulu DM. Lymphocyte to monocyte ratio as a screening tool for influenza. *PLoS Curr*. 2010;2:RRN1154. doi:10.1371/currents.rrn1154.
9. Vijapura AY, Pandya HP, Parmar B. Study of H1N1 cases admitted at tertiary care hospital with special reference to neutrophil-lymphocyte ratio and lymphocyte-monocyte ratio as a screening tool for suspected H1N1. *Int J Med Sci Public Health*. 2015;4(11):1565–8.
10. Tinh PT, Lan PT, Mahomed P, Nguyen TM. Epidemiological and clinical characteristics of patients who died from Influenza A (H1N1)pdm09 in Viet Nam. *Western Pac Surveill Response J*. 2012;3(1):6–11.
11. Unal S, Gökçe M, Aytaç-Elmas S, Karabulut E, Altan I, Ozkaya-Parlakay A, et al. Hematological consequences of pandemic influenza H1N1 infection: a single center experience. *Turk J Pediatr*. 2010;52(6):570–5.
12. Chan WL, Goh HK, Vasu A, Lim BL, Seow E. Experience of a screening centre for influenza A/H1N1: the first 50 days. *Emerg Med J*. 2011;28(1):18–24.
13. Shin DH, Han SK, Choi PC, Woo HY. Conventional blood tests in predicting influenza A (H1N1) rapidly. *Scand J Clin Lab Invest*. 2012;72(3):197–203.
14. Voudoukis E, Pappas A, Panoutsopoulos A, Xynos K, Rozi F, Giannakopoulou K, et al. Novel influenza A (H1N1) infection vs. common influenza-like illness: a prospective study. *Med Sci Monit*. 2011;17(4):185–8.
15. Coşkun O, Avci IY, Sener K, Yaman H, Ogur R, Bodur H, et al. Relative lymphopenia and monocytosis may be considered as a surrogate marker of pandemic influenza a (H1N1). *J Clin Virol*. 2010;47(4):388–9.
16. Cunha BA, Pherez FM, Schoch P. Diagnostic Importance of Relative Lymphopenia as a Marker of Swine Influenza (H1N1) in Adults. *Clin infect Dis*. 2009;49(9):1454–6.
17. Indavarapu A, Akinapelli A. Neutrophils to lymphocyte ratio as a screening tool for swine influenza. *Indian J Med Res*. 2011;134(3):389–91.

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Cite this article: Ballal P, Mahashur AP, Shruthi Rai P, Prabhudesai PP. Uncovering the diagnostic value of white blood cell profiles in H1N1 screening. *IP Indian J Immunol Respir Med* 2023;8(4):140-144.