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RESEARCH ARTICLE

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"Lymphocyte * Neutrophil" count decreased in SARS-CoV-2 Omicron patients in Shanghai with no significant change in CRP and SAA

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Abstract

Background: At present, there is a new variant Omicron BA.2 of SARS-CoV-2. In some previous studies, it was found that CBC, NLR, CRP, SAA, etc. in patients with SARS-CoV-2 had a series of changes, which were significantly correlated with the diagnosis and prognosis of patients. Therefore, in order to find specific diagnostic indicators, we explore the changes in these blood indicators and inflammatory indicators in patients with the SARS-CoV-2 Omicron.

Methods: A total of 127 Omicron confirmed patients who had visited fever clinic was selected as the positive group, and 75 Omicron excluded patients were selected as the negative group. We collected and analyzed the CBC, CRP, SAA test data, and clinical data of all subjects for analysis and statistics.

Results: WBC, NEU, LYM, EOS, PLT, PCT, LYM * NEU count compared with the negative group were significantly lower ($p < 0.05$); on the contrary, CNR were significantly higher ($p < 0.05$); The levels of CRP and SAA were not significantly different from those of the negative group ($p > 0.05$); the AUC of 0.781 for the diagnosis of LYM * NEU with an optimal cutoff value of 5.79, with a sensitivity and specificity of 68% and 73%, respectively, Youden index of 0.41, giving the best diagnostic performance.

Conclusion: The decreased LYM * NEU count can be used as the early, rapid, and accurate diagnostic indicator for Omicron. While due to the attenuated toxicity of BA.2 sublineage, CRP and SAA had no significance in the differential diagnosis of confirmed patients.

KEYWORDS
CRP, lymphocyte, neutrophil, omicron, SAA, SARS-CoV-2

1 | INTRODUCTION

The latest strong strain of SARS-CoV-2 Omicron attack Shanghai in late February 2022, caused a large area of human infection in the city, and according to the Shanghai Health and Wellness Commission, the cumulative number of infections has reached more than 600,000 by late May 2022. Researchers[1] compared the evolutionary characteristics of the genome of this new coronavirus with data from the Global Influence Sharing Database (GISAID) and showed that the viral genomes of newly infections in Shanghai all belonged to

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the BA.2 sublineage, which is a sublineage of the Omicron variant B.1.1.159. BA.2 included multiple sublineages and showed distinct patterns of geographic distribution. The absence of a significant difference in the distribution of the various mutations produced by the BA. 2 sublineage between severe and milder cases, which indicates that the severity of the infection may be mainly attributed to the complications caused by the patient's previous underlying diseases.

At present, most infections who were asymptomatic and mild diseases in Shanghai, the clinical manifestations of which are mainly fever, dry cough, and fatigue, lack specificity, so the clinic is based on RT-PCR for diagnosis confirmation, while hematological examination is currently the common method for clinical auxiliary diagnosis because of its easy, rapid, and economical operation. Neutrophil and lymphocyte-based indexes have been proposed as novel inflammatory markers in various clinical diseases including irritable bowel disease,[2] COVID-19 infection,[3] cardiac conditions,[4] and thyroiditis.[5] In addition, CRP-based markers are recognized as predictor of inflammation in diabetic nephropathy,[6] in hepatitis,[7] thyroiditis,[8] and in autoimmune liver conditions.[9] Thus, it can be hypothesized that LYM * NEU and CRP can be associated with SARS-CoV-2 infection with Omicron variant.

Based on the above research findings as well as the author's actual work, the data are hereby collected for research, aiming to explore the differences between the blood indicators, CRP, SAA of patients under infection with Omicron mutant strain and conventional influenza, expecting to find a hematological indicator with high sensitivity and specificity, so as to provide reference for early diagnosis and treatment.

2 | MATERIALS AND METHODS

2.1 | Sample collection

We selected 127 RT-PCR positive patients admitted to the fever clinic of Shanghai TCM-Integrated Hospital from March to May 2022 as the positive group, aged from 6 to 100 years, including 68 males and 59 females, with a median age of 67; In addition, 75 patients in fever clinic with RT-PCR negative in the same period were selected as the negative group, aged from 7 to 102 years, including 30 males and 45 females, with a median age of 60.《Protocol for the diagnosis and treatment of novel coronavirus pNEUmonia (Trial 9th Edition)》Omicron mutation: A confirmed case is one with a clear epidemiological history or typical clinical manifestations of COVID-19, and one of the following pathogenic or serological evidence (1) The novel coronavirus nucleic acid test is positive; (2) Both novel coronavirus specific IgM and IgG antibodies were positive in those not vaccinated with the novel coronavirus vaccine. This study has been approved by the hospital ethics committee.

2.2 | CRP and SAA measurement

The CBC(complete blood count) was measured by BC5180 blood cell analyzer and accompanying reagents manufactured by Mindray in Shenzhen, China, and the method was electrical impedance combined with laser. SAA and CRP assays were performed using a PA-800 specific protein analyzer and accompanying reagents manufactured by Lifotronic in Shenzhen, China, and the methods were scattering turbidimetry. The SARS-CoV-2 nucleic acid detection reagents were purchased from Biogerm Biotechnology company in Shanghai, China. The amplification instrument is ABI 7500, and the method is fluorescent quantitative PCR. 2 ml of the patient's venous whole blood was drawn and put into the anticoagulant blood collection vessel with dipotassium EDTA for CBC, CRP and SAA detection. All items should be operated in strict accordance with the operating procedures and reagent instructions, and indoor quality control testing should be carried out every day to ensure the testing quality.

2.3 | Statistical analysis

SPSS 24.0 software was used for data analysis, and GraphPad Prism 8 for drawing the Histogram. The measurement data conforming to the normal distribution is expressed in Mean ± SD, and the comparison between the positive and negative groups adopts *t* test; the measurement data that do not conform to the normal distribution are expressed in median and interquartile range(IQR), and the comparison between the two groups adopts Mann–Whitney *U* test. The ROC curve was also generated to determine the efficacy of different parameters in distinguishing SARS-CoV-2 patients from negative group. The area under the curve (AUC) was calculated. The 95% CI was calculated whenever appropriate, and a two-tailed $p < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Comparison of age, CBC, and inflammatory indexes between positive group and negative group

WBC, NEU, LYM, EOS, PLT count as well as PCT level, LYM*NEU were significantly lower ($p < 0.05$) in blood routine indexes compared with control group, CNR were significantly higher ($p < 0.05$); For the other indicators, the Mon, Bas, RBC count, HGB, HCT, MCV, MCH, MCHC, RDW-CV, PDW, MPV, and PLCR were not significantly different from the control group ($p > 0.05$); No significant differences were observed in the levels of inflammatory markers CRP and SAA compared with the control group ($p > 0.05$), which are shown in Table 1, Figure 1.

3.2 | Analysis of diagnostic efficacy of hematological indexes in positive group

The receiver operating characteristic curve (ROC) analysis showed that the AUCs of WBC, NEU, LYM, EOS, PLT, PCT, and LYM * NEU for the diagnosis of Omicron were 0.721, 0.675, 0.726, 0.646, 0.675, 0.665, and 0.781, respectively; The optimal cutoff values were 7.25,

TABLE 1 Comparison of CBC and inflammatory indexes between positive and negative groups

Parameters	SARS-CoV-2-positive group	SARS-CoV-2-negative group	*t/z*	*p* Value
n	127	75	–	–
age	67(51–80)	60(36–70)	−3.030	0.002
WBC($\times10^9$/L)	6(4.5–7.8)	8.6(5.8–12.3)	−5.251	<0.001
LYM($\times10^9$/L)	1.21(0.72–1.53)	1.56(1.27–1.90)	−5.363	<0.001
NEU($\times10^9$/L)	4.25(2.81–5.85)	6.5(3.67–9.81)	−4.162	<0.001
EOS($\times10^9$/L)	0.03(0.01–0.07)	0.05(0.02–0.13)	−3.487	<0.001
PLT($\times10^9$/L)	167(134–215)	206(161–259)	−4.164	<0.001
PCT(%)	0.16(0.12–0.19)	0.18(0.15–0.22)	−3.926	<0.001
HGB(g/L)	134.97 ± 17.82	134.25 ± 18.25	0.184	0.785
HCT(%)	38.77 ± 5.00	38.82 ± 5.00	1.000	0.947
MON($\times10^9$/L)	0.47(0.35–0.66)	0.52(0.41–0.74)	−1.911	0.056
BAS($\times10^9$/L)	0.01(0.00–0.01)	0.01(0.00–0.02)	−1.234	0.217
RBC($\times10^{12}$/L)	4.37(4.00–4.66)	4.41(3.97–4.68)	−0.321	0.748
MCV(fL)	90.4(87.4–93.3)	90.8(87.6–93.3)	−0.239	0.811
MCH(pg)	31.6(30.5–32.7)	31.4(30.1–32.2)	−0.873	0.382
MCHC(g/L)	348(340–357)	345(341–353)	−1.462	0.144
RDW-CV(%)	12.1(11.6–12.6)	12(11.6–12.7)	−0.087	0.930
PDW(%)	16.1(15.9–16.5)	16.1(15.8–16.3)	−1.790	0.073
PLCR(%)	20.2(15.4–25.8)	18.3(14.8–25)	−1.405	0.160
MPV(fL)	9(8.2–9.8)	8.7(8.2–9.5)	−1.385	0.166
LYM*NEU($\times10^{18}$/L^2)	3.94(2.60–7.21)	10.05(5.11–16.68)	−6.658	<0.001
n	122	75	-	-
CRP (mg/L)	3.00(2.00–8.00)	4.00(2.00–10.00)	−0.412	0.680
CNR	0.95(0.45–2.81)	0.66(0.37–1.73)	−2.019	0.043
n	87	75	-	-
SAA (mg/L)	28.79(15.88–56.65)	32.66(12.74–61.42)	−0.254	0.800

Abbreviations: BAS, basophils; CNR, C-reactive protein to neutrophil count ratio; CRP, C-reactive protein; EOS, eosinophils; HCT, hematocrit; HGB, hemoglobin; LYM*NEU, lymphocyte multiply by neutrophil; LYM, lymphocyte; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MON, monocyte; MPV, mean platelet volume; n, number; NEU, neutrophil; PCT, thrombocytocrit; PDW, platelet distribution width; PLCR, large platelet ratio; PLT, platelet; RBC, red blood cell; RDW-CV, red blood cell distribution width-coefficient of variation; SAA, serum amyloid A; WBC, white blood cell count.

6.36, 1.11, 0.03, 185.50, 0.17, and 5.79; The sensitivity was 68%, 80%, 47%, 47%, 60%, 63%, and 68%; specificity was 68%, 53%, 99%, 75%, 69%, 63%, and 73%, respectively; the Youden index was 0.36, 0.33, 0.39, 0.22, 0.29, 0.26, and 0.41, respectively. We can find that the LYM * NEU had an AUC of 0.781, an optimal cutoff value of 5.79, the sensitivity and specificity were 68% and 73%, respectively, as well as with a Youden index of 0.41, the diagnostic yield was the best, which are shown in Table 2, Figure 2.

4 | DISCUSSION

Since the outbreak of the epidemic in Wuhan in December 2019, novel coronavirus has spread rapidly throughout the country and even the world. In the past 3 years, the virus has been mutating in the process of transmission. Recently, with the Omicron mutant gradually replacing the delta strain, it has become a new public health threat. The recent outbreaks in Hong Kong and Shanghai in China were caused by the Omicron mutant strain, which spread rapidly, caused a serious threat to people's lives and health and also has a huge impact on the economy and society.

SARS-CoV-2 enters the host through the angiotensin converting enzyme 2(ACE2), similar to SARS-CoV-2 and other coronaviruses,[10] ACE2 is a membrane-bound peptidase that contains the N-terminal domain and extracellular catalytic site of most protein receptors,[11] and the protein S has two domains, S1 and S2, the S1 domain contributing to the linkage as it contains the RBD, and the S2 domain aiding viral fusion with the host cell.[12] The Omicron variant was identified in South Africa in November 2021 and assigned the VOC by World Health Organization at the same month, because of its high transmission rate, potential for immune evasion, and adverse effects on clinical diagnosis and treatment.[13] The Omicron variant

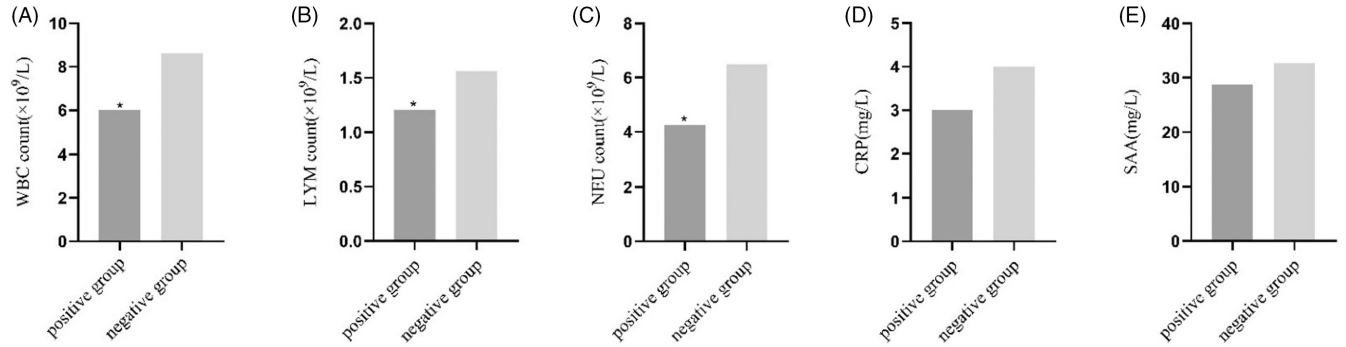

FIGURE 1 Comparison of CBC and inflammatory index between positive group and negative group The histogram (median and IQR) indicate the levels of WBC count (A), LYM count (B), NEU count (C), CRP(D) and SAA(E) between positive group and negative group. * $p < 0.05$

TABLE 2 Diagnostic efficacy of hematological indices

Variable	Cutoff value	AUC	Sensitivity (%)	Specificity (%)	Youden index	95%CI	*p* value
WBC($\times10^9$/L)	7.25	0.721	68	68	0.36	0.646~0.796	<0.001
NEU($\times10^9$/L)	6.36	0.675	80	53	0.33	0.595~0.756	<0.001
LYM($\times10^9$/L)	1.11	0.726	47	99	0.39	0.658~0.794	<0.001
EOS($\times10^9$/L)	0.03	0.646	47	75	0.22	0.567~0.725	<0.001
PLT($\times10^9$/L)	185.50	0.675	60	69	0.29	0.600~0.751	<0.001
PCT (%)	0.17	0.665	63	63	0.26	0.589~0.741	<0.001
LYM*NEU($\times10^{18}$/L^2)	5.79	0.781	68	73	0.41	0.715~0.846	<0.001

Abbreviations: AUC, area under the ROC curve; CI, confidence interval; Youden index, sensitivity +specificity 1.

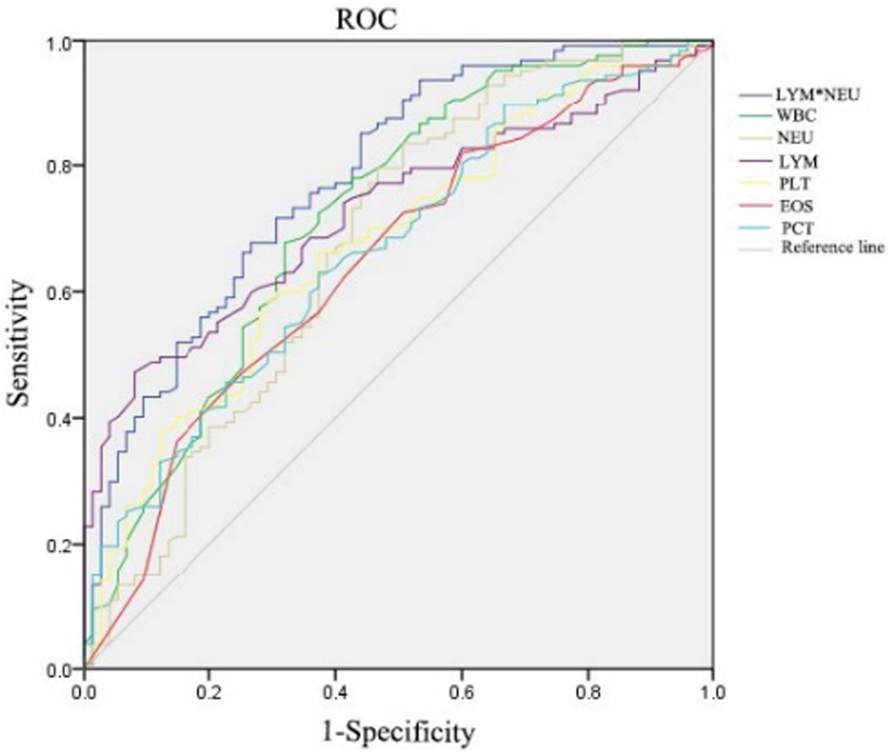

FIGURE 2 The ROC curve of hematological indexes in patients with SARS-CoV-2 Omicron

contains over 30 mutations in protein S and 15 mutations in the RBD alone, and the study posits that spike mutations facilitate its binding to the receptor, impairing binding to mAbs to evade the immune response.[14] Investigators[15]found that 24h after infection, the Omicron variant replicated 70-fold more efficiently than the original strain and the Delta variant in human bronchi and less efficiently

in human lung tissue than the original strain, which replicated 10-fold less. Another study[16]also demonstrated its faster replication in bronchi than other SARS-CoV-2 species (Alpha, Beta, Delta) and a lower replication capacity in human lungs. This may also explain the majority of the current Omicron infected population being predominantly milder. A study[17]has shed new light on the cause of a new round of high crown death data in Hong Kong, resulting from unvaccinated persons over the age of 60 years. The more complete comprehensive the vaccination, the greater, protective efficacy of the vaccine could reduce the rate of severe illness and case fatality. In view of the above, the reduction of Omicron's presenting symptoms has two factors, one is a decrease in the virulence of Omicron, and the second is because of the high vaccination rate, by of the end of May, the new crown vaccine in our country has been vaccinated over 3.3 billion doses.[18] However, while reducing virulence, a strong immune escape has emerged,[19–20] The vaccine is still an effective protective measure as far as the current situation is concerned, at the same time, the elderly and vulnerable people with basic diseases are the Omicron susceptible group.

In this study, the CBC and inflammatory indexes determined to infected Omicron group were compared with the negative group, and it was found that WBC, NEU, LYM, EOS, PLT, PCT, and LYM * NEU were all decreased compared with the negative group, combined with the AUC of LYM * NEU (0.781), which was most valuable for the differential diagnosis of Omicron, followed by the LYM (0.726), WBC (0.721) counts. The reduced level of LYM in Omicron patients may be due to the destruction of the patient's immune system by SARS-CoV-2, and the investigators found that the lymphopenia in critically ill patients was mainly associated with significantly decreased absolute counts of T cells, especially $CD8^+$ T cells, and the increased NLR was associated with severe SARS-CoV-2 illness as a useful prognostic factor[21]; Decreased LYM * WBC may serve as an early diagnostic indicator for SARS-CoV-2.[22] The reason for the poor diagnostic value of NEU (AUC 0.675) in this study is that this parameter may depend on different analytical stages of the disease or the type of population evaluated. Based on the high diagnostic value of LYM, the AUC of LYM * NEU was only 0.781, This reminds us that hematological parameters can be affected by many factors both in and out of the human body, so when using these parameters, the epidemiological history, clinical symptoms, and CT techniques should be combined to make a clinical diagnosis.

CRP as an inflammatory factor, when the body has acute infection, CRP combines with the pathogen choline phosphate or free calcium ions in serum to activate the body's immune system, stimulate complement and strengthen the phagocytosis of phagocytes, and eliminate pathogenic microorganisms invading the body. It is often markedly elevated for 6~8 h in response to tissue injury or inflammation.[23] It has been widely used in clinic, for example, the increase in CLR (C-reactive protein to lmyphosphate count ratio) is specific for the diagnosis of thyroiditis and hepatitis.[7,8] CAR (C-reactive protein to serum albumin ratio) has high sensitivity and specificity in the diagnosis of diabetes nephropathy.[6] CRP has been suggested to be associated with respiratory function (PaO_2/FiO_2), is a predictor of respiratory failure[24], and it's elevation is widely used as an inflammatory factor in SARS-CoV-2 infection.[25–27]In our study, we found that CNR was increased compared with the negative group, and the difference was statistically significant ($p<0.05$). In view of the decrease of neutrophils, CRP may be increased to some extent, but it was not obvious.

Elevated SAA is mainly seen in viral infection, cardiovascular disease, transplant rejection, etc., and the sensitivity is higher than that of CRP, which is produced in large amounts when bacteria or viruses infect humans under the stimulation of inflammatory cytokines. SAA can be rapidly elevated by approximately 1000-fold within 4~6 h of pathogen infection.[28] SAA and associated pathologies may be a long-term risk of SARS-CoV-2 infection.[29] Another study[30] indicated SAA significantly higher in patients with SARS-CoV-2. In the present study, we found that CRP and SAA were not statistically significant in the experimental group after Omicron infection compared with the control ($p>0.05$), suggesting that these two indicators of inflammation may not be suitable for the auxiliary diagnosis of Omicron, it is speculated that compared with the original strain and other mutants that have been found, the toxicity of Omicron is reduced, and the inflammatory and immune responses mediated by Omicron are also weakened.

5 | CONCLUSION

CBC is the most widely used laboratory test for patients with symptoms suggestive of colds and is readily available even in primary hospitals, and a reduced LYM * NEU count can be used as the most helpful diagnostic indicator in Omicron suspected patients.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the author upon reasonable request.

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