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ORIGINAL ARTICLE

Application of Peripheral Blood Lymphocyte Count in Prediction of the Presence of Atypical Lymphocytes

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SUMMARY

Background: **Atypical lymphocytes (AL), or reactive lymphocyte, exist in peripheral blood when stimulated by viral infection, drugs, inflammatory signals or allergens. Studies have shown that specific changes in peripheral blood (PB) analysis can predict morphological changes in blood cells. The objective of this study was to explore the value of the peripheral blood lymphocyte count in predicting the presence of AL.**
Methods: **One hundred ninety-nine outpatients were selected from Beijing Chao-Yang Hospital, Capital Medical University from January to April 2015 and underwent manual cell classification with evaluation of complete clinical data. The results of manual classification of peripheral blood leukocytes and peripheral blood routine analysis were assessed, and the correlation between peripheral blood lymphocyte counts and presence of atypical lymphocytes evaluated using receiver operating characteristic (ROC) curves for each subject.**
Results: **Peripheral blood lymphocytes ≥ 2.375 x 10^9/L was found to be the optimal cutoff point for predicting atypical lymphocytes. The area under the curve (AUC), 95% confidence interval (CI), sensitivity and specificity were 0.7984, 0.7121 - 0.8846, 68.42%, and 82.8%, respectively, while the accuracy was moderate. When the proportion of peripheral blood lymphocytes was greater than 35.90%, the AUC, 95% CI, sensitivity, and specificity were 0.8729, 0.8092 - 0.9366, 89.47%, and 76.34%, respectively, while the accuracy was moderate.**
Conclusions: **The peripheral blood lymphocyte count of a patient has good predictive value for the existence of atypical lymphocytes, which is helpful for clinical diagnosis.**
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KEY WORDS

cutoff point, atypical lymphocytes (AL), receiver operating characteristic (ROC) curve, sensitivity, specificity

INTRODUCTION

Atypical lymphocytes (AL), or reactive lymphocytes, exist in peripheral blood when stimulated by viral infection, drugs, inflammatory signals or allergens. AL generally show morphological variations and blast transformation in T lymphocytes, which are characterized by a large cell body, increased cytoplasm, blastogenesis or enhanced presence of cytoplasmic basophilia. AL are occasionally found in the blood of healthy people, but generally account for less than 2% of leukocytes, the cell count will only increase as a result of virus infec-

tion or other factors. A proportion greater than 5% has clinical significance, and an increase of 10% - 20% is most valuable for diagnosis [1]. A blood count analyzer can assist multi-sample and multi-parameter examination of a large number of blood samples, greatly improving efficiency. However, current blood count analyzers can only be used for preliminary screening, and microscopic examination is essential when suspicious conditions are encountered [2]. Studies have shown that specific changes in peripheral blood (PB) analysis can predict morphological changes in blood cells, for example, basophil count higher than 1.2% can be used as a marker to predict the existence of atypical lymphocytes [3], and a peripheral blood lymphocyte count higher than 7 x 10^9/L has good predictive value for lymphoblastic diseases [4]. However, there are limited comparable studies performed in China. Therefore, the objective of this study was to explore the value of the peripheral blood lymphocyte count in predicting the presence of AL.

MATERIALS AND METHODS

Study setting and design

From January to April 2015, 199 outpatients who underwent manual cell classification with complete clinical data were selected from Beijing Chao-Yang Hospital, Capital Medical University, including 104 males and 95 females; the patients were aged 15 - 91 years with an average age of 45.04 ± 18.39. Due to the difference in reference range, child cases were not included in this study. Clinical symptoms included viral infection, fever, leukopenia, pneumonia, respiratory tract infection, lymphadenitis, and lymph node enlargement. The existence of atypical lymphocytes was confirmed by morphological examination of peripheral blood, and the patients were divided into a non-atypical lymphocyte group (93 cases) and an atypical lymphocyte group (106 cases).

Morphology review

The microscopic PB smear examination was conducted basically as described by Lv Juan et al. [5]. Venous blood (2 mL) was collected from the patients into EDTA-K2 anti-coagulant tubes, and the peripheral blood routine test was completed by automatic blood analyzer (Sysmex XE-2100, Sysmex Corporation, Japan) within 30 minutes. A blood smear with distinct head, body, and tail was prepared and dried at room temperature. After Wright-Giemsa staining (Baso Diagnostics Inc., Zhuhai), morphological evaluation was mainly performed by four of the authors (YTZ, HZ, GBM, XZW), all full-time hematopathologists with at least 15 years of working experience, using a binocular microscope (Olympus CX-41, Japan) with oil (1000 x).

Statistical analysis

Graphpad-Prism 5.0 software was used for data analysis. The normally distributed measurement data were represented by ($\bar{x} \pm s$), and the non-normally distributed data were represented by the median. Significant difference was assumed at $p < 0.05$. The receiver operating characteristic (ROC) curve of the subjects was drawn by Graphpad-Prism 5.0 statistical software to determine the optimal diagnostic cutoff point for peripheral blood lymphocytes, and the area under the curve (AUC), sensitivity, specificity, and Youden's index were calculated. An AUC of > 0.9, 0.7 - 0.9, and 0.5 - 0.7 indicates high, medium, and low accuracy, respectively [6].

RESULTS

Morphological evaluation of atypical lymphocytes

According to the morphological characteristics, atypical lymphocytes can be divided into 3 types (as shown in Figure 1): type I: vacuole type or plasma cell like (Figure 1a); type II: irregular type or monocyte type (Figure 1b); type III: immature type (Figure 1c). The morphology of peripheral blood atypical lymphocytes covered these three categories. The atypical lymphocytes accounted for more than 5% in 89 cases, and more than 10% in 37 cases; the highest proportion was 53%, and the median was 7.5%.

Prediction of the numbers and proportions of peripheral blood lymphocytes for predicting AL

The ROC curve showed the optimal cutoff point, AUC, 95% CI, sensitivity, specificity, and accuracy in the proportion and number of peripheral blood lymphocytes for predicting atypical lymphocytes > 0, > 5%, and > 10% by morphological evaluation is shown in Figure 2a - f and Table 1. The AUC was at least 0.7114. Especially, a proportion of peripheral blood lymphocytes ≥ 35.90% was the optimal cutoff point for predicting atypical lymphocytes > 10%, while the AUC, 95% CI, sensitivity, and specificity were 0.8729, 0.8092 - 0.9366, 89.47%, and 76.34%, respectively, while the accuracy was moderate (as shown in Figure 2e).

DISCUSSION

The causes of atypical lymphocytes include: infection (EB virus, cytomegalovirus, rubella virus, herpes simplex virus, etc.), drug and toxin factors, immune factors, radiation factors, hormone-related autoimmune diseases, malignant diseases, idiopathic diseases, and transplant rejection [7]. In disease diagnosis, the number of atypical lymphocytes increases significantly in viral infection, especially in EB virus infection, but can also be found in bacterial infection or other viral infections, and even in healthy people. Therefore, EB virus infection is considered when the proportion of atypical lymphocytes is higher than 10% [8]. Virus infection mainly causes changes in the number and morphology of lymphocytes. Some virus infections cause mainly B

Table 1. Prediction of the count of peripheral blood lymphocytes for predicting AL.

	AL > 0		AL > 5%		AL > 10%	
	LYMPH (%)	LYMPH (#)	LYMPH (%)	LYMPH (#)	LYMPH (%)	LYMPH (#)
Figure	2a	2b	2c	2d	2e	2f
Cutoff	≥ 36.30%	≥ 2.330 x 10^9/L	≥ 35.90%	≥ 2.330 x 10^9/L	≥ 35.90%	≥ 2.375 x 10^9/L
AUC	0.7212	0.7114	0.7722	0.7309	0.8729	0.7984
95% CI	0.6504 - 0.7920	0.6407 - 0.7822	0.7035 - 0.8409	0.6592 - 0.8027	0.8092 - 0.9366	0.7121 - 0.8846
Sensitivity	63.55%	52.34%	74.44%	57.78%	89.47%	68.42%
Specificity	76.34%	81.72%	77.42%	81.72%	76.34%	82.80%
Accuracy	moderate	moderate	moderate	moderate	moderate	moderate

AL - atypical lymphocytes.
LYMPH (%) - the proportion of peripheral blood lymphocytes.
LYMPH (#) - the number of peripheral blood lymphocytes.

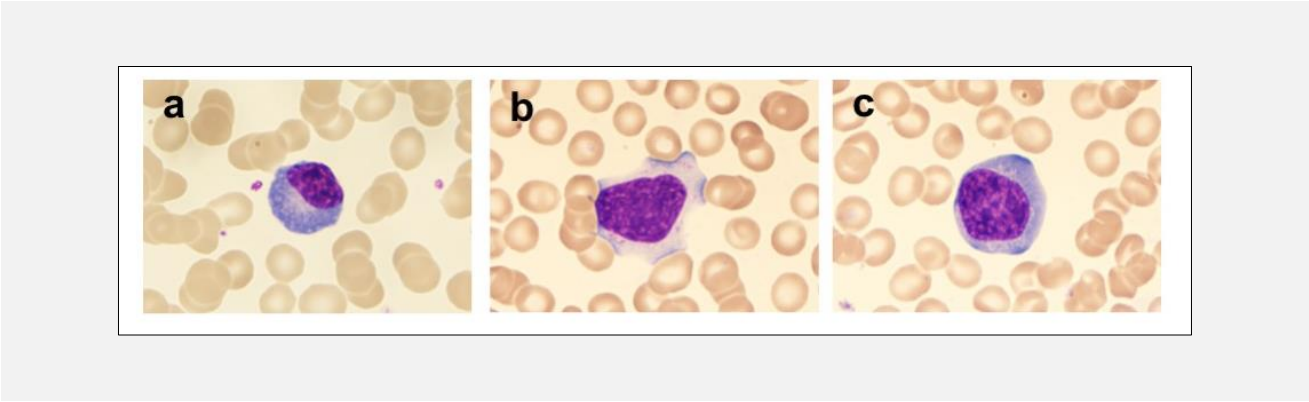

Figure 1. Morphological classification of atypical lymphocytes in peripheral blood.

lymphocyte reactions at first and then strong reactivity of T lymphocytes, resulting in atypical lymphocytes observed in peripheral blood smears. Clinically, due to the difficulty and time-consuming nature of virus culture, the clinical manifestations of some viral infections are very complex and difficult to specify. Although viral antibody measurements can provide clues, this has certain limitations. Meanwhile, the existence and number of atypical lymphoid cells can be determined according to the changes of VCS (volume, conductivity, and scatter) parameters of lymphocytes in blood routine analysis, but the instrument can only be used for preliminary screening, and microscope re-examination should be performed in complex situations to avoid omissions [9]. Exploration of the potential association between the blood routine indexes and atypical lymphoid cells, in order to prompt timely morphological examination of blood cells, is therefore of great significance to guide clinical diagnosis. It was reported that the sensitivity and specificity of peripheral blood basophils reaching 1.2% in predicting the existence of atypical lymphocytes were 73.4% and 81.9%, respectively [3]. At present, there are limited related studies based on Chinese populations. Considering the potentially different characteristics of patients from different regions and populations, it is necessary to evaluate and verify the optimal diagnostic cutoff points and diagnostic values of the numbers of different cells in peripheral blood in the Chinese population.

Preliminary results of this study showed that the number of lymphocytes is most closely associated with the existence of atypical lymphocytes. The ROC curve in this study showed that the AUC was at least 0.7114, indicating that the count of peripheral blood lymphocytes is associated with the presence of atypical lymphocytes. The optimum cutoff point for the proportion of peripheral blood lymphocytes was further determined as 35.90% - the sensitivity, specificity, and Youden's index of which in predicting the existence of atypical lymphocytes were 89.47%, 76.34% and 0.6581, respec-

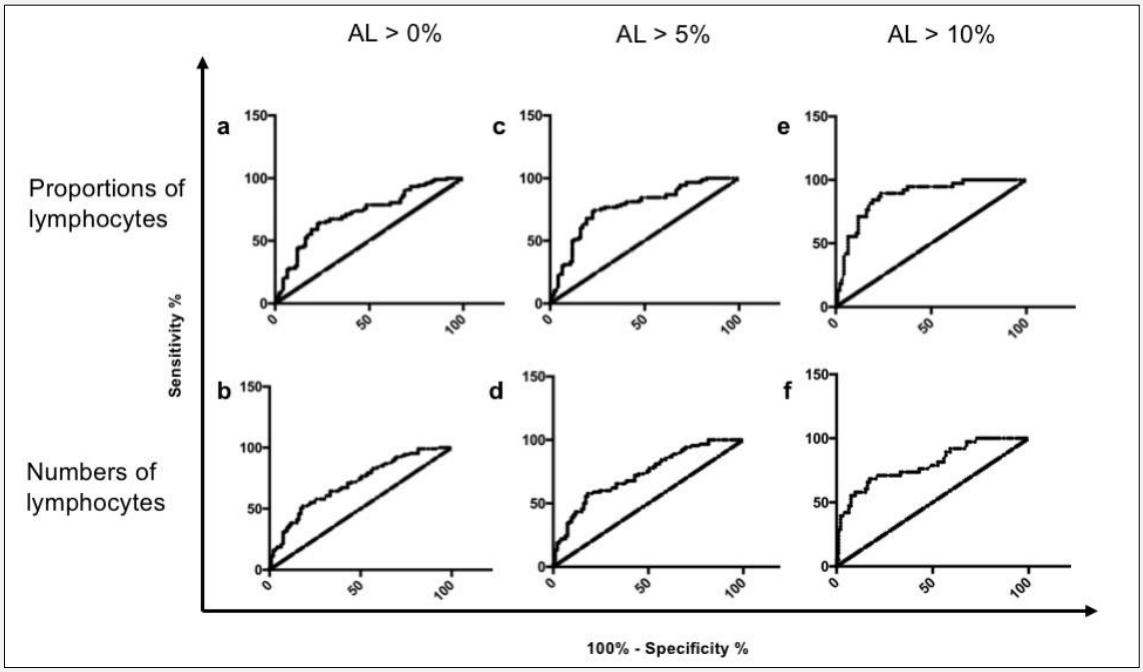

Figure 2. ROC curve of the peripheral blood lymphocyte count for predicting AL.

a. Prediction of atypical lymphocytes by the proportion of peripheral blood lymphocytes. b. Prediction of atypical lymphocytes by the number of peripheral blood lymphocytes. c. Prediction of atypical lymphocytes > 5% by the proportion of peripheral blood lymphocytes. d. Prediction of atypical lymphocytes > 5% by the number of peripheral blood lymphocytes. e. Prediction of atypical lymphocytes > 10% by the proportion of peripheral blood lymphocytes. f. Prediction of atypical lymphocytes > 10% by the number of peripheral blood lymphocytes.

tively, and the AUC was 0.8729. The optimum cutoff point for the number of peripheral blood lymphocytes was determined as 2.375 x 10^9/L - the sensitivity, specificity, and Youden index of which in predicting the existence of atypical lymphocytes was 68.42%, 82.8%, and 0.5122, respectively, and the AUC was 0.7984. This study showed that the counts of lymphocytes can be used to predict atypical lymphocytes. In the reference ranges for differential white blood cell count in normal adults, the reference range for proportion of peripheral blood lymphocytes is 20% - 50%, and the reference range for the number of peripheral blood lymphocytes is 1.1 - 3.2 x 10^9/L. However, according to the results in this study, when the proportion of peripheral blood lymphocytes is more than 35.9%, or the number is more than 2.375 x 10^9/L, morphological examination indicates the existence of atypical lymphocytes. These two cutoff points are close to the upper limit of the reference range and have high sensitivity and specificity. Based on the existing investigations, when blood routine values reach the above cutoff points, it is suggested that synthetic analysis combining other clinical features of the patient is carried out, including microscopic examination, in order to judge the situation of the patient more accurately, better guide clinical treatment, and prevent omission of the examination information of the patient.

Meanwhile, as a diagnostic basis, the proportion of lymphocytes has a larger AUC than the number of lymphocytes, indicating that the proportion of lymphocytes has higher accuracy and better indication as a basis for diagnosis. Compared with numbers, proportions can better rule out the effect of changing white blood cell counts on the total number of lymphocytes. The proportion represents the relative change of the number of lymphocytes, which is more accurate. Therefore, the proportion of lymphocytes can better predict the existence of atypical lymphocytes than the number.

Since this study was a retrospective study, however, incomplete data for some patients was inevitable. Therefore, further expansion of the sample size would help to reduce the effect of selection bias on the conclusions. In addition, blood cell morphology testing is a microscopic observation of blood smears using the naked eye. Therefore, the subjectivity of the results can lead to a certain bias in the results even if an experienced technician strictly follows the standard operating procedures. Thus, in order to better ensure the reliability of results,

the atypical lymphocyte group was further divided into more than 5% and more than 10% for statistical analysis, to minimize the statistical grouping bias caused by different judging standards of atypical lymphocytes.

CONCLUSION

In summary, the counts of peripheral blood lymphocytes can predict the existence of atypical lymphocytes to a significant extent, which can prompt the laboratory physician to timely examine blood cell morphology of patients and can provide important information regarding clinically undetected virus infections. The results of this study should prompt laboratory physicians to further improve the standard of microscopic re-examination of atypical lymphocytes, in order to avoid clinical omissions [3].

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Declaration of Interest:
The authors declare no conflict of interest.

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