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The morphological changes of monocytes in peripheral blood as a potential indicator for predicting active pulmonary tuberculosis

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ABSTRACT

Background: Monocytes play a crucial role in immune response against Mycobacterium tuberculosis infection. The purpose of this current study was to investigate the morphology present on monocytes in peripheral blood from patients with active pulmonary tuberculosis (APTB) and the laboratory performance of the changes for discriminating cases from normal healthy subjects (NHS).

Method: A total of 71 peripheral blood samples from patients with APTB, and 65 samples from NHS were analyzed. The mean monocyte volume with its distribution width and mean monocyte conductivity as well as monocyte light scatter were detected by VCS technology used on the LH750 hematology analyzer. Correlations of these changes with the serum cytokine level in the immune alterations were further evaluated. The Receiver operating characteristic curve (ROC) analysis was used to highlight the clinical implication.

Results: In APTB patients, the mean monocyte volume showed significant difference associated with an evident elevation in the mean monocyte volume distribution width compared to those in NHS. Furthermore, the mean monocyte volume had positive relationship with the serum level of interleukine-1β response to M. tuberculosis infection. Simultaneous measurement of the mean monocyte volume and its distribution width was able to distinguish active infection with an excellent sensitivity of 84.5% and specificity of 90.5% comparable to those obtained from pro-inflammatory cytokine interleukine-6 identifying APTB with great accuracy.

Conclusion: The morphological changes of monocytes particular increased mean volume may be a potential indicator to predict active tuberculosis infection.

1. Introduction

Tuberculosis (TB), caused by Mycobacterium tuberculosis, is a serious infectious disease commonly affects the lungs [1]. Interactions between the host and M. tuberculosis largely determine the development and outcome of active pulmonary tuberculosis (APTB), and monocyte-derived macrophages play a critical role in granulomatous responses against M. tuberculosis infection [2–4].

Despite the mechanism of newly-recruited monocytes inside the granuloma is still not fully understood, recent studies have shown that in vitro monocytes from the periphery could come into contact with mycobacterial or cellular products and become infected with M. tuberculosis to differentiate into macrophages through expression of key cell markers, cytokine release, anti-mycobacterial activity and T-cell proliferation [5,6]. Given the central role of monocytes in the induction of immune responses, their morphological changes in peripheral blood might to be expected to reflect the state of immunity to TB infection [7]. The VCS technology used on the LH750 hematology analyzer is able to generate the differential count based solely on cellular morphology, using neither chemical reactions nor fluorescence. The volume or cell size is measured directly by impedance. The conductivity reflecting the internal cellular density is measured by the conduction of radio frequency waves across the cell, and the laser light scatter gives direct information regarding cytoplasmic granularity and nuclear complexity. This analysis is performed by obtaining three parameters simultaneously that are directly correlated to cellular morphology [8–10].

In this study, we investigate the morphology present on monocytes in peripheral blood from APTB patients, and the correlation of these changes with the serum level in the immune alteration was further evaluated. In addition, we hypothesize the potential usefulness of these

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changes for discriminating APTB patents from normal healthy subjects (NHS).

2. Materials and methods

2.1. Case selection

Patients with APTB were recruited from the Sixth People Hospital of Nantong, China from April 2014 to August 2015. Active pulmonary TB was confirmed based on typical clinical symptoms, chest X-radiography, positive Ziehl-Neelsen acid fast bacilli staining, positive Lowenstein-Jensen slants bacterial culture for sputum, apparent anti-TB drugs efficacy [11]. Patients were HIV-negative, and those with a positive bacterial culture that was not M. tuberculosis, or those with hematological disorder were excluded from this study. Normal healthy subjects (NHS) were randomly selected from the Second Affiliated Hospital of Nantong University, China from October 2014 to May 2015. All individuals underwent regular health check up, with following inclusion criteria: (1) without any signs of infection; (2) with CBC count and differential data within normal limits; (3) without HIV infection. This research was approved by the Ethics of Committee of the Sixth People Hospital of Nantong (Jiangsu, China). Informed written consent was obtained from all participants.

2.2. Hematological data collection

Cell population data (CPD) of peripheral blood collected in ethylene-diamine tetra-acetic acid (EDTA) containing tubes were performed on a five-part differential hematology analyzer (Beckman Coulter LH750, Fullerton, Unite States). Such parameters reflect the mean monocyte volume with its standard deviation (monocyte volume distribution width) and the mean monocyte conductivity, as well as monocyte light scatter [12]. Cell population data measurement was subject to strict quality assurance procedures according to the manufacturer's instructions. Stability of VCS parameters showed not any significant changes within 4 h after specimen collection.

2.3. Cytokine analysis by ELISA

Blood was collected using Heparin blood collection tubes (BD Bioscience, San Diego, CA), and serum separation was performed to quantify cytokines. Serum levels of the cytokines IL-1β and IL-6 were measured by enzyme-linked immuosorbent assay (ELISA) method. Cytokine concentrations were determined using a standard curve obtained from the standards provided by the manufacturer with each kit, and the results were expressed as ng/L. The measuring ranges of the assays were from 1.0 to 20 ng/L for IL-1β and 0.4 ng/L for IL-6. All samples were run in duplicate and the assays were performed following the supplier's instruction (Senjiasen, Nanjing, Jiangsu, China).

2.4. Statistical analyses

All Analyses were performed using SPSS software, version 18.0 (SPSS, Chicago, IL). Results were expressed as the mean ± standard deviation (SD). Comparisons between 2 means were performed by using the Student's test. Correlations were determined using Spearman's rank correlation coefficients. Diagnostic properties of each test were estimated by receiver operator characteristic (ROC) curve analysis. Graphpad Prism 5.01 (Graphpad Software, CA, USA) was for plotting the data. Statistically significant differences were determined using $P < 0.05$.

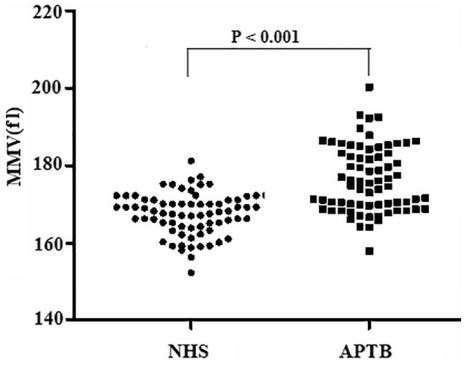

Fig. 1. The mean monocyte volume (MMV) in active pulmonary tuberculosis (APTB) patients showed significant difference ($P < 0.001$) compared to normal healthy subjects controls (NHS).

3. Results

3.1. Changes of the monocyte VCS parameters in APTB patients

In this retrospectively study, a total of 71 APTB patients (mean age: 39.4y, range 19–71 y, male: female ratio: 1.1: 1) including 41 cases with M. tuberculosis positive culture and 30 with negative culture, and 65 normal healthy subjects (mean age: 38.6 y, range 20–72 y, male: female ratio 1.1: 1) were enrolled. Of all VCS parameters analyzed on the hematology instruments using VCS technology, the mean monocyte volume showed significant difference (176.44 ± 8.51 fl versus 167.59 ± 5.48 fl, P value = 0.000) associated with an evident elevation in the mean monocyte volume distribution width (21.12 ± 3.15 versus 18.16 ± 2.18, P value = 0.000) when compared to those in NHS (Fig. 1). However, there were no statistically significant differences of the mean monocyte conductivity (117.14 ± 5.84 versus 116.21 ± 5.29, P value = 00.154) and the mean monocyte scatter (85.88 ± 5.36 versus 87.96 ± 5.29, P value = 0.057) observed between two groups. It is conceivable that the morphological changes of monocytes due to M. tuberculosis infection tend to be larger in sizes as shown with increased mean monocyte volume with its distribution width (Table 1).

In addition, the mean monocyte volume had positive relationship with the serum level of cytokine IL-1β produced in M. tuberculosis infected monocytes [5,13] (correlation coefficient = 0. 484, P value = 0.000), where an increase in this serum cytokine was evident with an elevation in the cell size (Fig. 2). Cytokine IL-1β may participate in the alterations observed in the monocyte changes in morphology. Thus these changes led us to propose that the increased cell volume may reflect the immune response specific to active tuberculosis infection.

3.2. Morphological changes of monocyte for discriminating between APTB and NHS group

In this current study, we have identified that APTB patients presented with increased mean monocyte volume, indicating a systemic activation state of monocytes during disease development. The receiver operating characteristic (ROC) curve analysis was applied to evaluate the discriminatory ability of the monocyte VCS parameters between APTB and NHS group. The values of the area under the curve (AUC) of these parameters are shown in Table 1. Among all VCS parameters, the mean monocyte volume was strongest discriminator between APTB and NHS with an AUC of 0.897. Using the cutoff value of 172.60 fl, the mean monocyte volume achieved a relatively good sensitivity of 80 3% and specificity of 87 3% comparable to a sensitivity of 81 7% and a specificity of 90 1% obtained from pro-inflammatory cytokine IL-6 at the cutoff value of 3.95 ng/L to distinguish active infection from NHS.