นิพนธ์ต้นฉบับ

Flow cytometry pattern in the diagnosis of cryptococcal meningitis in HIV-infected patients

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Abstract:

Flow cytometry pattern in the diagnosis of cryptococcal meningitis in HIV-infected patients

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The counting of leukocytes and erythrocytes in cerebrospinal fluid (CSF) is still performed microscopically, e.g., using a chamber, in most laboratories. This requires sufficient practical experience, is time-consuming, and may constitute a problem in emergency diagnostics. Recently, following advances in flow cytometry technology, some reports have mentioned the use of flow cytometry analyzer as a good alternative for CSF cell count. In this article, we report our experience with the flow cytometry pattern of cryptococcus meningitis in 4 HIV-infected patients. In general, the parameters showed a lymphocytic profile with basophilia. The flow cytometry pattern presented in this study is the first report. Although there are some limitations due to the nature of a preliminary report, we decided to report these data to encourage other research groups to study this topic.

Key words: *flow cytometry, cryptococcal meningitis*

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บทคัดย่อ:

การนับเม็ดเลือดแดงและเม็ดเลือดขาวในน้ำไขสันหลังด้วยการนับผ่านกล้องจุลทรรศน์เป็นวิธีที่ใช้กันโดยทั่วไปในปัจจุบันในห้องปฏิบัติการทางการแพทย์ อย่างไรก็ตามวิธีดังกล่าวต้องใช้นักเทคนิคการแพทย์ที่มีความชำนาญ และใช้เวลาการตรวจที่นาน ปัจจุบันมีการพัฒนาเทคนิค flow cytometry เป็นอย่างมากและมีการริเริ่มนำมาใช้ในการตรวจน้ำไขสันหลัง ในรายงานนี้ได้รายงานผลการศึกษาเบื้องต้นถึงรูปแบบของ flow cytometry จากผู้ป่วย HIV 4 รายที่ติดเชื้อ cryptococcal meningitis พบลักษณะร่วมคือ lymphocytic profile with basophilia รายงานนี้นับว่าเป็นรายงานแรกในการศึกษาเชิงดังกล่าว ผู้นิพนธ์หวังว่าผลการศึกษานี้จะเป็นประโยชน์ที่จะเป็นข้อมูลเบื้องต้นสำหรับผู้อื่นในการศึกษาต่อไปในอนาคต

Introduction

The counting of leukocytes and erythrocytes in cerebrospinal fluid (CSF) is still performed microscopically, e.g., using a chamber, in most laboratories. This requires sufficient practical experience, is time-consuming, and may constitute a problem in emergency diagnostics[1]. Recently, due to advances in the flow cytometry technology, some reports have mention the use of flow cytometry analyzer as a good alternative for a CSF cell count[2]. However, there is no report concerning the application of flow cytometry to the fungal cell.

The use of a flow cytometry system for a CSF cell count can be useful in the diagnosis of neurological disorders since it can provide rapid results[1-2]. Furthermore, in a setting where the rate of HIV transmission is high as in Thailand, it seems to be safer than the classical microscopic method. In this article, we report our experience with the flow cytometry pattern of cryptococcus meningitis in 4 HIV-infected patients.

Materials and methods

Flow cytometry analyzer

The flow cytometry analyzer used in this study was a Technicon H*3[3]. This machine is an automated hematology analyzer for *in vitro* diagnostic use in clinical laboratories. The system is based on the principle of light cytometry. Each sample was consecutively processed; the first count coming from the cellular channel was discarded as it was used as primer and the final count was obtained by calculation. The acceptable sensitivity and specificity of this flow cytometer ranges from 0 up to 100,000 particles/μL.

The Technicon H*3 system provides the operator with the results of a complete blood count and a five part differential for each specimen tested. Both CBC parameters (WBC : White blood cell count, RBC : Red blood cell count, HGB : Hemoglobin concentration, HCT : Hematocrit, MCV : Mean corpuscular volume, MCH : Mean corpuscular hemoglobin, MCHC : Mean corpuscular hemoglobin concentration, RDW : Red cell distribution width, HDW : Hemoglobin distribution width, Plt : Platelet count, MPV : Mean platelet volume, PDW : Platelet distribution width, PCT : Plateletcrit) and differential parameters (Neut %: Neutrophil percent, Lymph%: Lymphocyte percent, Mono%: Monocyte percent, Eos%: Eosinophile percent, Baso%: Basophil percent, LUC%: Large unstained cells percent, Neut# :Neutrophil count , lymph#: Lymphocyte count, Mono#: Monocyte count, Eos#: Eosinophil count, Baso#: Basophil count) can be obtained in each analysis.

From our comparative study of CSF count by routine manual and Technicon H*3 systems, the correlation coefficients for WBC count, lymphocyte count and neutrophil count were equal to 0.984, 0.835 and 0.855 respectively[4].

Cryptococcal meningitis specimens

All 4 cryptococcal meningitis CSF specimens mentioned in this study were derived from 4 HIV seropositive hospitalized patients in King Chulalongkorn Memorial Hospital. All of them were diagnosed to have crytococcal meningitis, confirmed by the classical indian ink technique. The leftover CSF, from laboratory analysis, of the first collection before any treatment of each patient was collected and sent for further flow cytometry analysis at the Clinical Microscopic Laboratory, Faculty of Allied Health Science, Chulalongkorn University. All analyses were performed by only one practitioner to avoid observer variation.

Statistical analysis

The data of each subject were collected then analyzed by descriptive statistics. The averages of each flow cytometry parameter were calculated and presented. All statistical analyses were performed using SPSS10.0 for Windows program.

Results

The flow cytometry parameters for each subject are presented in table 1.

Discussion

Cryptococcosis is a fungal infection that is potentially deadly for, and common among, AIDS patients in the United States and worldwide. Subacute meningitis and meningoencephalitis are typical, clinically[5]. Presently, the gold standard for diagnosis is culture. However, that method is expensive, therefore basic tests such as CSF culture and Indian ink stain are widely used. In general the positive rate upto 76 % can be detected by Indian ink staining compared to culture[6].

However, the classical microscopic method has a risk of contact with the infectious agent within the body fluid. Therefore, the use of the flow cytometry technology to cope with this problem has been widely discussed[1]. According to a recent study[7], the use of the flow cytometry to analyze the cellular parameters was acceptable. It is recommended as an alternative method to the manual microscopic method.

Here, we present a preliminary report on the flow cytometry parameters from analysis of 4 samples from HIV-infected patients with definitive diagnosis of cryptococcal menigitis. Furthermore, we use the first collected samples before any treatment.

Table 1 Flow cytometry parameters

parameters	Case 1	Case 2	Case 3	Case 4	average
WBC(x 10^3/μL)	0.21	0.62	0.81	0.67	0.58 ± 0.26
RBC(x 10^6/μL)	0	0	0	0	0
HGB(g/dL)	0	0	0	0	0
HCT(%)	0	0	0	0	0
MCV(fL)	69.2	62.3	71	68.8	67.63 ± 3.76
MCH(pg)	0	0	0	0	0
MCHC(g/dL)	0	0	0	0	0
RDW(%)	23	42.1	0	1.5	16.65 ± 19.96
HDW(g/dL)	4.83	5.66	0	0	2.62 ± 3.05
Plt(x 10^3/μL)	1	1	1	1	1
MPV(fL)	6.2	4.1	5.8	3.8	4.98 ± 1.20
RDW(%)	72.9	76.6	83.3	106.9	84.93 ± 15.27
PCT(%)	0	0	0	0	0
%neutrophil	6	0.6	23.1	2.4	8.02 ± 10.30
%lymphocyte	88.6	99.2	41.7	94.3	80.95 ± 26.52
%monocyte	3.6	0.2	0.9	0	1.18 ± 1.66
%eosinophil	0	0	33.3	0.9	8.55 ± 16.51
%basophil	23.2	14.8	3.4	2.4	10.95 ± 9.92
LUC	1.8	0	0.9	0	0.68 ± 0.86
MPXI*	-34.9	64.8	-25.5	-16.4	-3.0 ± 45.83

*myeloperoxidase index

In general, the analysis showed a lymphocytic profile with basophilia. The flow cytometry pattern presented in this study is the first report. Although there is some limitation due to the nature of a preliminary report, we decided to report these data to encourage other research groups to study this topic.

Conclusion

In this article, we report our experience with the flow cytometry pattern of cryptococcus meningitis in 4 HIV-infected patients. In general, the parameters showed a lymphocytic profile with basophilia.

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