Determination of monocyte count by hematological analyzers, manual method and flow cytometry in polish population

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Abstract

The aim of the study was to compare the absolute and relative monocyte count obtained by microscopic method (M), flow cytometry (FC) and the automated hematological analyzers (HA) as well as to validate of reference ranges of monocytes count in polish population. Blood samples from 84 healthy adults were analyzed. The cell counts determined by three HA: two HMX and one Cell-Dyn were confronted with M and FC. The counts exceeding 800/ul were found in 4.8%, 5.9%, 15.5% and 13.1% patient's blood samples assessed by: M, FC, HMx and Cell-Dyn, respectively. The difference between both HA and M was significant ($p<0.05$). FC detection of relative count provided the closest results to M but difference between the means was significant. The staining anti CD45 and anti CD14 monoclonal antibodies gives the best agreement with the both HA than with M. All examined methods revealed significant percentage of monocytes above the upper limit of the reference range accepted as 8%. Our preliminary data indicate that the upper limit of the reference range of monocytes should be higher. There is a great need for cross-sectional population study to establish new reference range for monocytes in order to appropriately manage individuals with infection diseases and hematological disturbances.

Key words: *monocytes, counting, different methods*

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Introduction

The blood monocytes are young cells that already possess migratory, chemotactic, and phagocytic activities, as well as receptors for IgG Fc-domains (FcγR) and C3b complement. They undergo differentiation (at least one day) to become multifunctional tissue macrophages. Thus they represent the circulating macrophage population and when they are ready for migration into a tissue, the change of phenotype occurs in response to factors encountered in specific tissue after migration. The analysis of blood cell count, including monocyte enumeration, is a routine diagnostic procedure in medicine. Clinical practice, supported by several publications, suggests existing discrepancy between normal values of monocyte count in comparison to those accepted as reference one's [1-4]. Polish textbooks give reference value for monocytes as 2-8% of leukocyte population and absolute number in range 200-800/l [5, 6]. It is concordant with the range 0-7% given by Wallach [7] and Ravel [8] and 0-800/μl by Tietz [9]. The discrepancy exists also between monocyte count from different methods their enumeration [10-12]. M was considered as a gold standard routinely used to validate differentials obtained by other methods [3]. However, nowadays FC enumeration of monocytes stained with monoclonal antibodies has been proposed as a possible new reference method for monocyte counting [10].

The aim of the study was to compare the absolute and relative monocyte count obtained by microscopic method (M), flow cytometry (FC) and automated hematological

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analyzers (HA) as well as to assessed validity of generally accepted the reference ranges of monocyte count.

Material and methods

Blood samples (EDTA-K3) from 84 healthy blood donors: 42 men (mean age 25.9±7.52) and 42 women (mean age 24.3±5.78) were analyzed. Pre-analytical and analytical phases were performed according to the guidelines of the IFCC and Polish Society of Cytometry. The morphologic analysis on a total of 500 leucocytes was performed by two independent experienced technologists in accordance with the protocol of the NCCLS. The number of monocytes was determined by three HA: two different instruments of HMX (Beckman-Coulter, USA) and one Cell-Dyn (Abbott Diagnostics, USA) and was confronted with FC performed with an Epics XL (Beckman-Coulter, USA). FC analysis assessed expression CD14, CD45, CD36, HLA-DR and CD4 without CD3 on mononuclear blood cells. After 20 min incubation 100 µl of blood with 20 µl of two different antibody combinations conjugated with FITC and PE erythrocytes were lysed. Appropriate isotypic control was always included.

For statistical analysis a software program (Statistica vs. 6.0) was used. Descriptive statistics and linear regression analysis, along with the Student's t test were used. Chi square test was used to compare the distribution of high and normal monocyte count obtained by different instruments. Results with $p<0.05$ were considered as statistically significant.

Results

The results of precision study revealed that the highest precision was provided by FC (variability index 4,96%) vs 7,05% (M) and 6,05% (HMX). Table 1 shows ranges and the mean values of relative and absolute monocyte count obtained by different methods. Monocytes count exceeding 800/ul was found in 4.76%, 5.9%, 15.5% and 13.1% blood samples studied by M, FC, HMx and Cell-Dyn, respectively. The difference between both HA and M was significant (HMx vs M χ^2=4.6345; $p<0.05$, Cell-Dyn vs. M χ^2=3,8367; $p<0.05$). Moreover, in 2 out of 84 (2.4%) samples assessed by M and FC, in 3 samples out of 84 (3.4%) assessed by HMx and in 6 out of 51 (11.2%) assessed by Cell-Dyn the absolute number exceeded 1000

Table 1. Comparison of the relative and absolute values of monocyte count estimated by manual, automated (HMx and Cell-Dyn analysers) and flow cytometry methods

n	Method	Relative number (%)			Absolute number/µl	
		range min-max	mean±SD	correlation coeffficient	range min-max	mean±SD
84	CD14+CD45+	3.6-15.4	7.4±2.37	0.61	179-1540	499.3±221.3
	microscopy	3.7-11.2	6.7±2.43		169-1770	452.2±221.4
	p		<0.05	<0.001		NS
84	CD14+CD45+	3.6-15.4	7.4±2.37	0.82		499.3±221.3
	HMX	5.5-18.3	9.7±2.73		223-1810	610.4±240.31
	p		<0.001	<0.001		<0.001
84	HMX	5.5-18.3	9.7±2.73	0.68	223-1810	610.4±240.31
	microscopy	3.7-11.2	6.7±2.43		169-1770	452.2±221.4
	p		<0.001	p<0.001		<0.05
51	CD14+CD45+	4.9-13.4	7.0±1.9	0.75		485.1±208.1
	CELL-DYN	3.5-13.2	8.3±2.31		282-2110	616.1±342.2
	p		<0.01	<0.001		<0.05
51	CELL-DYN	3.5-13.2	8.3±2.31	0.47	282-2110	616.1±342.2
	microscopy	3.9-10.9	7.0±2.35		169-1770	431.1±191.4
	p		<0.05	<0.05		<0.05
21	HMx	5.2-17.1	9.1±2.3	0.9	223-1810	598.4±223.3
	CELL-DYN	3.5-13.2	8.5±2.31		282-2110	607.1±242.1
	p		NS	<0.001		NS

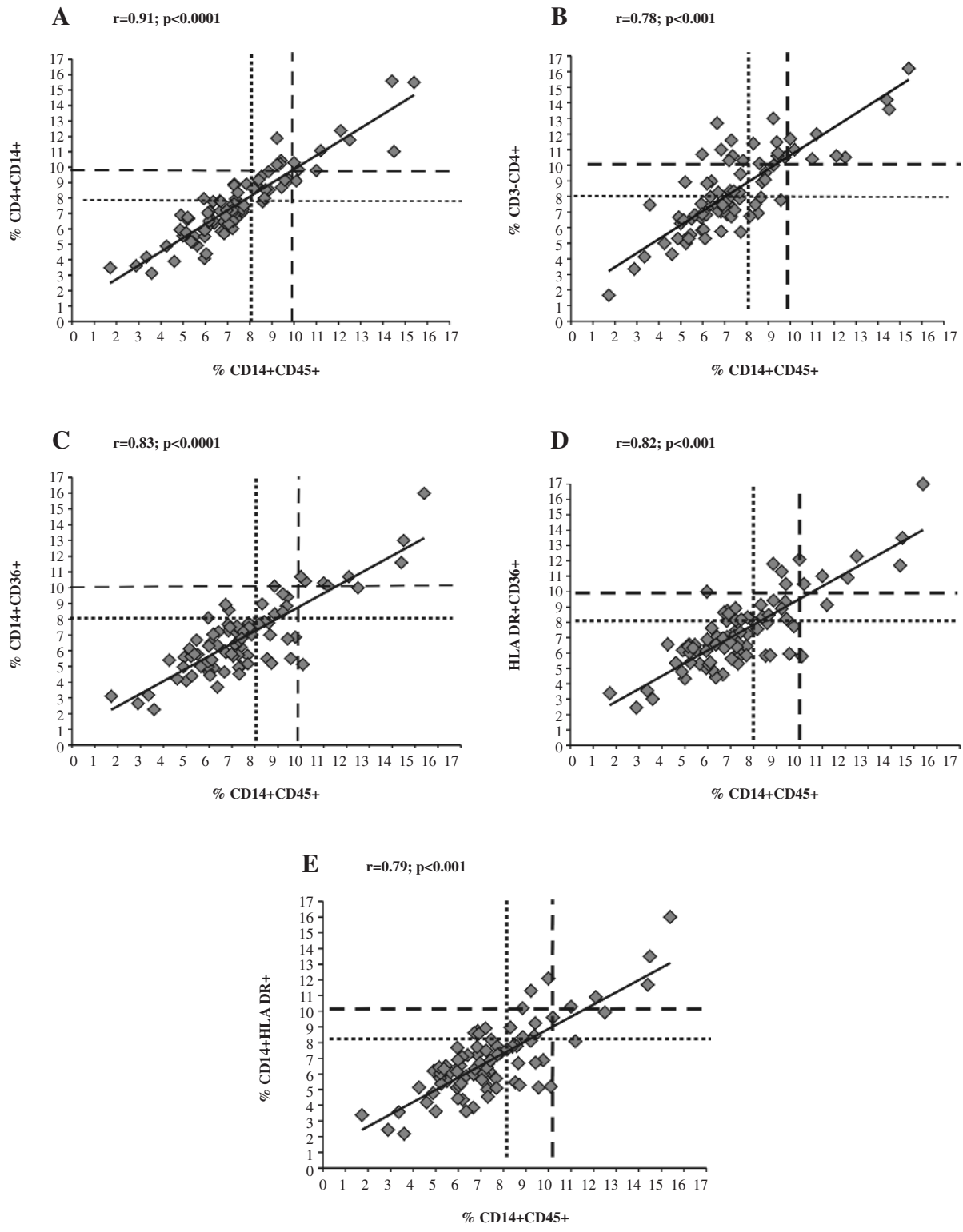

Fig. 1. Correlation between percentage of cells CD14+CD45+ and CD4+CD14+ (A), CD3- CD4+ (B), CD14+CD36+ (C), HLA-DR+CD36+(D) and CD14+HLA DR+ (E). r – correlation coefficient: dropped and broken line – upper limit 8% and 10% accordingly

Table 2. Frequency of relative monocyte count exceeding 8% and 10% by different methods

Percentage of monocytes	Number of patients with monocytosis by different methods				
	microscope	MoAB CD14+/CD45+	MoAB CD4+/CD45+	HMX	CELL-DYN
>8	17/84 (20.2%)	26/84 (30.9%)	26/84 (30.9%)	57/84 (67.9%)	26/51 (50.9%)
>8>10	12/84 (14%)	17/84 (20.2%)	15/84 (17.8%)	34/84 (40.5%)	15/51 (31.4%)
>10	5/84 (6.0%)	9/84 (10.7)	11/84 (13.1%)	23/84 (27.4%)	10/51 (19.6%)
>8 by all the methods	11/84 (13.1%)				

cells/µl. FC detection of relative monocytes number (CD45+CD14+) provided the closest results with regard to M but difference between the means was significant. The significant difference was observed also between the both HA vs. M and FC. Results from HMx and Cell-Dyn were not significantly different. The correlations between results obtained from all examined systems were statistically significant (Tab. 1) but determination of the monocyte count after dual staining anti CD45 and CD14 gives better agreement with the both HA than with M. All immunophenotypes correlated very well with classical monocyte immunophenotype CD14+CD45+ (Fig. 1). All methods revealed a number of samples with monocyte count above the upper limit of the reference range (Tab. 2). In 11 cases out of 84 (13.1%) higher number of monocytes was confirmed by all methods. Highly significant differences in the frequency of results exceeding 8% were seen in all of the three methods (χ^2=68.8115; p<0.001). HA showed significantly higher frequency of high monocyte count comparing to M (χ^2=38.6429; p<0.00001). FC and M showed no statistical difference in distribution of results exceeding 8%. Number of monocytes >10% was more frequently noticed by FC then by M (χ^2=3.8345; p<0.05). Higher monocyte percentage was more frequently revealed by HA in comparison to both FC and M (p<0. 0001). Very close results with no statistical difference between means, very good correlation (r=0.82; p<0.001) and almost identical yield of results exceeding upper limit of reference values was obtained by two identical HMX (data not shown).

Discussion

The microscopic analysis has been considered for a long time as a gold standard in monocyte enumeration. However, there are a lot of suggestions that FC should be regarded as an acceptable alternative. This method seems to be especially usefull because of HA have difficulties in differentiating monocytes from some lymphocytes and granulocytes. The overestimated count of the monocytes may be caused by the presence of immature forms of granulocytes, virus infected lymphocytes, large variant lymphocytes, blasts, Sezary cells, hairy cells, leukemia cells etc [12, 13]. These facts stresses the importance of the microscopic method, despite its lack of precision [12]. From presented analysis it can be concluded that FC could be a reference method in the evaluation of the differential count of leucocytes, including monocytes. FC is the most precise method, even if the coefficient of variation of HA is very similar. However, due to high costs of monoclonal antibodies and a flow cytometer we may accept that the values provided by HA are sufficiently precise to recommend for the routine clinical use. The opportunity for a clinician to request a microscopic examination must be preserved, because the clinician's knowledge of the patient's history, physical findings and therapy may indicate the possibility to discover an abnormality not apparent from the instrument results alone. Our preliminary data indicate that the upper limit of the reference range of monocytes should be higher. In patient's without any symptoms of disease a number of monocytes over references value is misleading to physician and may suggests chronic inflammatory disease, parasitic infection, tuberculosis or viral infection. For this reason, there is a great need for cross-sectional population study to establish new reference range for monocytes. The standardization criteria of pre-analytical and analytical phases should assure good reproducibility among laboratories so that the obtained reference ranges may be useful for interlaboratory comparison of results [3, 7]. The attention should be also paid to the instruments and the brand of monoclonal antibodies that may represent an inevitable cause of variability [10, 14]. Due to several factors including genetics, sex, age, and altitude adopting reference values from the past or from other populations might be misleading [13].

References

1. Kasili EG, Cardwell CL, Taylor JR (1969): Leucocyte counts on blood donors in Nairobi. East Afr Med J 46: 676-679.
2. Taylor MR, Holland CV, Spencer R, et al. (1997): Haematological reference ranges for schoolchildren. Clin Lab Haematol 19: 1-15.

3. Wintrobe MMG, Lee R (1999): Wintrobe's Clinical Hematology. The Williams&Wilkins Co, USA, pp. 34-35.
4. Saxena S, Wong ET (1990): Heterogeneity of common hematologic parameters among racial, ethnic, and gender subgroups. Arch Pathol Lab Med 114: 715-719.
5. Dembińska-Kieć A, Naskalski JW (1998): Diagnostyka Laboratoryjna z Elementami Biochemii Klinicznej. (ed) Urban & Partner. Wrocław, pp. 440-441.
6. Mariańska B, Fabijańska-Mitek J, Windyga J (2003): Badania Laboratoryjne w Hematologii. PZWL, Warszawa 34-35.
7. Wallach J (1996): Interpretation of Diagnostic Tests. Ed. Little, Brown and Co, Boston, New York, Toronto, London, pp. 340.
8. Ravel R (1995): Clinical Laboratory Medicine. Clinical Application of Laboratory Data. Ed. Mosby-Year Book. USA, pp. 123-124.
9. Tietz NW (1995): Clinical Guide To Laboratory Tests. Ed. Saunders WB, Philadelphia, pp. 321-322.
10. Hubl W, Tlustos L, Erath A, et al. (1996): Proposed reference method for peripheral-blood monocyte counting using fluorescence-labelled monoclonal antibodies. Cytometry 26: 69-74.
11. Hubl W, Andert S, Erath A, et al. (1991): Evaluation of automated basophil counting by using fluorescence-labelled monoclonal antibodies. J Clin Lab Anal 10: 177-183.
12. Goossens W, Van Hove L, Verwilghen RL (1991): Monocyte counting: discrepancies in results obtained with different automated instruments. J Clin Pathol 44: 224-227.
13. Ezeilo GC (1972): Non-genetic neutropenia in Africans. Lancet 2: 1003-1004.
14. Morillon M, Maslin J, De Pina JJ, et al. (1999): Evaluation of the monocyte counting by the ABX Vega. Comparison with the manual method and fluoro-flow cytometry. Hematol Cell Ther 41: 47-50.