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LEUKOCYTE POPULATIONS DETECTION IN YOUNG ATHLETES IN RESTING PHASE BASED ON SCATTER PROPERTIES USING A FLOW CYTOMETRIC APPROACH

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Abstract Complete blood count analysis has a major importance as a first laboratory evaluation. In particular data obtained from complete blood count are relevant to correctly diagnose and follow up different medical conditions. In athletes, medical examinations and laboratory test are mandatory, especially when a sportive event will soon take place. Different automatic techniques are used to evaluate percentages of different leukocyte populations with a high accuracy. Flow cytometry represents a technology that is mainly used to diagnose different lymphoproliferative and myeloproliferative neoplastic disorders. In this study 127 samples from clinically healthy young athletes were collected and analyzed with both flow cytometry and automatic method to evaluate the usefulness of flow cytometry in detecting different leukocyte populations based on their scatter properties. Results showed that flow cytometry is a reliable technique having showed a high accuracy for different cell populations. Intra class correlation coefficient was >0.80 for all populations showing a high correlation between the two methods. However, a higher number of cases, the involvement of other automatic techniques are mandatory to confirm these results.

Key words: flow cytometry, leukocytes, populations, athletes

Introduction

Complete blood count (CBC) is usually the first step toward a correct diagnosis to different medical conditions. All data gained by this examination can help clinical practitioners to better understand the origin of the clinical problems. In human medicine this exam is usually performed to detect different medical conditions and also for follow up purposes in cases of neoplastic disorders, especially when the leukocyte formula is affected (Seo & Lee, 2022; Ahmed et al., 2020; Juchnowicz et al., 2023; Agnello et al., 2021; Lassale et al., 2018). In athletes the CBC is an important exam that should take place periodically to observe changes that could have happened prior and after athletic events (Mairbäurl, 2013; Bachero-Mena et al., 2017; Wedin & Henriksson, 2020). Many parameters of complete blood count can change during exercise. Indeed, it was observed that red blood cells, hemoglobin concentration and the hematocrit values increase progressively during exercise, reaching the highest value during maximum exertion and then a linear decrease is observed (Ciekot-Sołtysiak et al., 2024) Each peripheral blood cell leukocyte populations can be examined by the classical method using microscopy. This technique has been used for many years, but nowadays the CBC is performed also by automatic techniques that have showed a high accuracy. However, automatic techniques have to be validated prior to their use in daily practice. To do such, other, already automatic validated techniques have to be used to compare results.

Flow cytometry represents a relatively new technique that can be used to identify different leukocyte populations using its scatter properties and antibodies. This type of machinery has been used for many years now in human and veterinary medicine. Mainly, flow cytometry is used to diagnose peripheral blood neoplastic disorders such as lymphoma and leukemia, allergies, Ki67 also known as Kiel 67 which is a nuclear protein strictly associated and used as a cell proliferation marker, cell apoptosis, minimal residual disease, DNA content and other conditions (Morse et al., 1994; Rütgen et al., 2021; Kim & Sederstrom, 2015; Mao et al., 2023; Wlodkowic et al., 2009; Wlodkowic et al., 2011; Boumiza et al., 2005; Otto et al., 2016). Research conducted lately in animals shows that flow cytometry can be used also to identify and stage solid tumors such as mast cell tumors in dogs (Sulce et al., 2018). However, the reliability of this technique in solid tumors has to be further investigated. The main advantage of flow cytometry is that cells populations can be analyzed for the presence of different antigens in their surface at the same time (Robinson et al., 2023; Drescher et al., 2021; Sanders & Mourant, 2023). On the other hand also, single cells can be analyzed and sorted anytime if needed (Telford, 2023; Mattanovich & Borth, 2006; Atajanov et al., 2018; Box et al., 2020; Hervieu et al., 2022). On the contrary other techniques such as western blot and histopathology cannot give such information, taking into consideration also the time needed to provide results.

Taking into consideration all of the above mentioned capacities we hypothesized that flow cytometry can be used to correctly detect different leukocyte populations in young athletes using only its scatter properties. The aim of this study was to evaluate the reliability of flow cytometry in identifying leukocyte cell populations in young athletes using cell morphological properties.

Material and methods

The experiment was conducted with the permission of the ethical committee for research of the University of Sports of Tirana/Albania (protocol number 167/1/2024).

Sampling

Young athletes aging between 19–23 years old in resting phase were chosen for this study. A total of 127 athletes were included in the study. One sample corresponds to one individual. Athletes were included in the study only if they met the following criteria: clinically healthy, vaccinated following the national program of vaccination, no chronic diseases, no clinical signs of any disease at the moment of the peripheral blood collection, presence of a medical statement that they are completely healthy. Peripheral blood collection was conducted at the cephalic vein by a professional, while maximum efforts were made to minimize distress following the best practices.

Flow Cytometry analyzes

Samples were collected and deposit in Ehtylentetracetat 2.5 ml tubes. Samples were stored in refrigerated conditions in 4°C. In all cases analysis were performed within 24h from collection in order obtain optimum results avoiding any cell deformation or damage. Concentration of cells was determined using an Attune NxT flow cytometer (Thermo Fisher Scientific). A determined quantity of 50 µl (concentration 1×10^{6}) was placed in flow cytometry tubes. A lysis step of 15 minutes took place immediately after placement in order to destroy the red blood cell integrity. Cells were than centrifuged at 1200 rpm × 5 min, supernatant was discarded and a washing step using phosphate saline buffer (PBS) was performed. Cells were then resuspended in 200 µl of PBS and acquisition in the cytometer took place. A total of at least 1×10^{6} cells were acquired in order to provide reliable results on cell populations. Propidium iodide was used in the second channel in order to exclude debris from the analysis. Individual gates were designed for each cell population. Thus, data regarding percentages for Granulocytes, Monocytes and Lymphocytes were collected for all samples.

Automated analyses

Peripheral blood samples collected were analyzed by an automatic technique using SYSMEX XN-550 fully automated machinery. All samples were analyzed within 24h from blood collections and all results were provided 2 hours after all samples were analyzed.

Statistical analyses

Data obtained from Flow Cytometry and the automatic technique were summarized and descriptive statistics were done. Intra class correlation (ICC) coefficient was performed in order to observe the agreement between flow cytometry and the automatic technique using the automatic technique as the reference method. Two way mixed model and the definition of absolute agreement was used to calculate the ICC for single measures. The ICC was interpreted as poor if its 95% confidence interval is less than 0.5, moderate if between 0.5 and 0.75, good if between 0.75 and 0.9 and excellent if above 0.90 (Koo & Li, 2016). Statistical analyses was performed using SPSS version 25 (IBM,SPSS Inc., Chicago IL).

Results

In total one hundred and twenty seven samples were collected from healthy athletes for this preliminary study. Automated and Flow Cytometry techniques were used to analyze all samples. Statistical analyses were made based on percentages of each population taken into consideration. Interclass correlation coefficient showed that

flow cytometry and automated technique have a great agreement especially for Granulocytes and Lymphocytes. The level of agreement for Monocytes can be considered as moderate to good. Descriptive statistic P value and interclass correlation coefficient are presented in table 1.

Table 1. Descriptive statistics for Granulocytes, Monocytes and Lymphocytes evaluated by automated technique and flow cytometry. Interclass correlation coefficient is showed along with other data

Leukocytes	Technique	Mean (%)	Minimum (%)	Maximum (%)	ICC	P value
Granulocytes	Automated	58.326	35.2	74.4		
	Flow Cytometry	58.422	34.7	72.7	0.981	<0.001
Monocytes	Automated	7.434	5.2	16.0		
	Flow Cytometry	7.110	5.1	15.3	0.834	<0.001
Lymphocytes	Automated	31.148	17	47.1		
	Flow Cytometry	31.142	16.8	49.2	0.982	<0.001

In table 2 and 3 data regarding age, gender, place of living (rural or urban) and type of sport (individual or team) are presented

Table 2. Data regarding the age of the athletes involved in the study

Parameter	Average	Minimum	Maximum	SD
Age	19.67	19	23	0.895

Table 3. Data regarding place of living, gender and type of sport of athletes involved in the study

Place of living	Percentage	Gender	Percentage	Type of sport	Percentage
Urban	72.44	Male	70.86	Individual	62.99
Rural	27.56	Female	29.14	Team	37.01

In figure 1 data designation and gating strategy of different gates on flow cytometry in order to calculate each cell populations is presented.

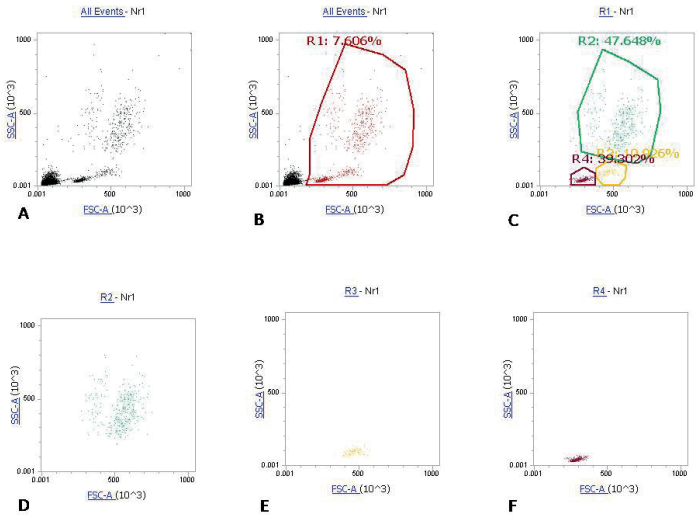

Figure 1. Gating strategy followed to evaluate each leukocyte population. (A) Dot plot representing all populations including debris, (B) All population leukocyte gating excluding debris, (C) Designation of gates for each leukocyte populations, (D) Activation of Granulocytes gate, (E) Activation and evaluation of Monocytes gate, (F) Activation of Lymphocytes gate

Discussion

Flow cytometry appears a reliable technique when the leukocyte populations identification is considered. Flow cytometry showed a high interclass correlation coefficient with the classical automated technique confirming almost the same results. Scatter properties of the machine were able to correctly identify the different leukocyte populations with a high accuracy. A high interclass correlation coefficient was observed for Granulocytes and Lymphocytes. Regarding Monocytes flow cytometry tend to underestimate their number when compared with the automated technique. This discordance can be due to the scatter properties presented by Monocytes in different stages of their activation. Moreover, in some cases it was difficult to design the proper gate for Monocytes since sometimes large Lymphocytes can enter to the gate by altering the percentages. As showed in figure 1 gates of Monocytes and Lymphocytes are located very near to each other causing time to time changes in their respective percentages. However, in the majority of cases gate designation was done properly and populations were well separated from each other. Moreover, in total results obtained from this study can be considered as satisfactory even if there are some limitations. The low number of caseload and missing the usage of other techniques such as microscopy and cell sorter can be considered as a big limitation for this study.

Limitations of the study

It has to be mentioned that the study has several limitations. Taking into consideration that this study is focused on the comparison of two different techniques the number of cases can be considered as relatively low. Moreover, the lack of antibody usage can slightly affect the results obtained during this investigation. Furthermore,

the use of a cell sorter can provide a larger information on the purity of the cell populations on each gate of analyses taken into consideration to obtain the different percentages.

Conclusions

Flow cytometry showed a high reliability for Lymphocytes and Granulocytes and moderate for Monocytes. These results showed that both techniques have a great agreement between each other demonstrating that flow cytometry can be routinely used to perform regular complete blood count in different situations.

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