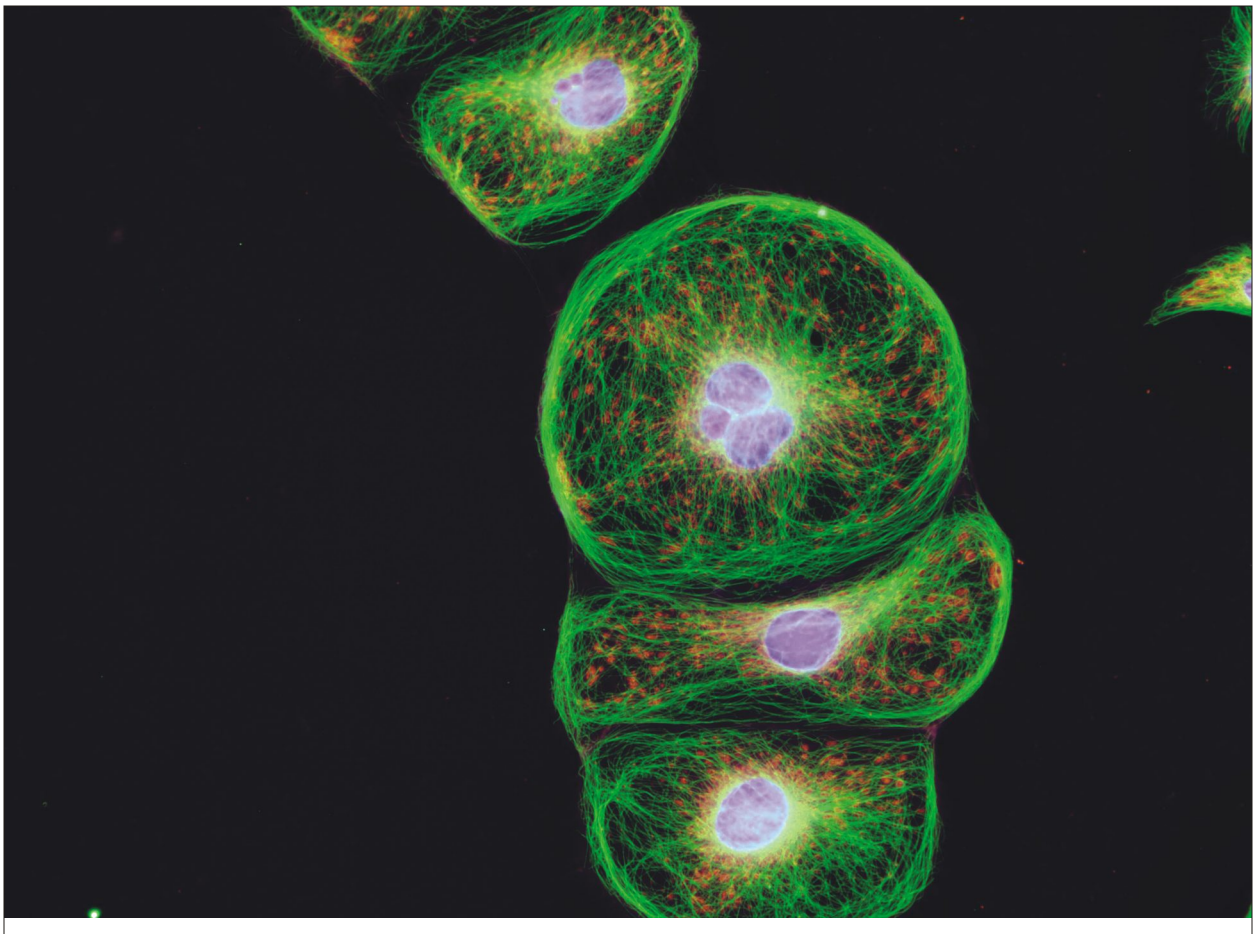

Asian Journal of Cell Biology

ISSN 1814-0068

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Asian Journal of Cell Biology

ISSN 1814-0068
DOI: 10.3923/ajcb.2022.1.9

Research Article
Peripheral Blood Leukocyte Cells Variation and their Involvement in the Immune-Inflammatory Response of Patients with Bronchial Asthma

[1,2]Cyrille Alode Vodounon

[1]Laboratory of Biochemistry and Immunology, National University of Sciences, Technologies, Engineering and Mathematics, ENS-Natitingou, BP: 72 Natitingou, Republic of Benin
[2]Department of Biochemistry, Institute of Fundamental Medicine and Biology, Kazan (Volga Region) Federal University, Kazan, Russian Federation

Abstract

Background and Objective: The onset of various diseases and especially disabling diseases such as bronchial asthma is often associated with quantitative and qualitative changes in leukocytes. The research aimed to study the features of leukocytes in bronchial asthma patients. **Materials and Methods:** The study was based on the count of certain leukocyte cells in the peripheral blood of relatively safe donors (30) and bronchial asthma patients (109), including 15 intermittent asthma, 38 mild persistent, 26 moderate persistent and 30 severe persistent asthmatics. Blood smears were used to count the number of leukocytes in donors using the garaeva camera. Lymphocytes were isolated with the zonal centrifugation method and were cultured under standard conditions. **Results:** The results showed a significant increase in the neutrophil and eosinophil in asthmatics compared to relatively safe donors and this increase was offset by a decrease in lymphocyte and monocyte. The correlational analysis method showed that there is an inverse relationship of dependence between counted lymphocytes and the degree of the disease severity ($r = -0.3$, $p = 0.04$, $R_2 = 0.0734$, $n = 49$) *in vivo*. In contrast to *in vitro*, based on slowed cell growth in the medium culture of patients with severe disease, lymphocytes from mild and moderate asthmatics showed a proliferative activity. **Conclusion:** According to the obtained results, it can be said that an estimation of certain figurative elements in the peripheral blood of patients with asthma would provide information on the actors involved in the initiation of the immune-inflammatory response in asthmatics.

Key words: Leukocytes, bronchial asthma, diseases disabling, smear blood, immune-inflammatory, peripheral blood, patients, quantitative, qualitative estimation

Citation: Vodounon, C.A., 2022. Peripheral blood leukocyte cells variation and their involvement in the immune-inflammatory response of patients with bronchial asthma. Asian J. Cell Biol., 17: 1-9.

Corresponding Author: Cyrille Alode Vodounon, Laboratory of Biochemistry and Immunology, National University of Sciences, Technologies, Engineering and Mathematics/ENS-Natitingou BP: 72 Natitingou, Republic of Benin Tel: 00229 96668870

Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The pathogenesis of Bronchial Asthma (AB) is defined as a specific inflammation process in the bronchial wall where immunocompetent cells play a key role[1,2].

Thus, lymphocytes like eosinophils, neutrophils and monocytes play a prominent role in inducing the perpetuation of the asthmatic inflammatory response[3-6]. They are also involved in the inflammatory process behind the development of airway hyperresponsiveness and bronchial remodeling[7,8]. This pathophysiological scheme has recently been complemented by the recent discovery of Innate Lymphoid Cells (ILCs)[9] and Th17 lymphocytes[10,11] which have led to the consideration of cells as a major player in the induction of type 2 adaptive responses and the establishment of AB[12,13]. Changes in the level of these cells in the blood of patients may indicate activation of the immune system in response to inflammation, or disease and reveal the functional status of the patient. It has been shown that the emergence of various diseases and particularly disabling diseases like AB is often associated with quantitative and qualitative changes in leukocytes. Therefore, the detection in peripheral blood of AB patients of a variation in leukocyte levels or individual leukocyte types may be used as diagnostic and prognostic tests, as markers or even as therapeutic target[14], in AB treatment.

Programmed cell death, characterized by a specific morphological pattern, is one of the mechanisms that ensure the control of cell homeostasis during the immune response. It should be noted that during AB, the majority of cells infiltrated into the airway resist apoptosis and it is assumed that the persistence of airway inflammation depends on the increased survival of these cells in the bronchial mucosa. Lavinskiene *et al.*[15] suggested that allergic asthma patients have decreased apoptosis of peripheral blood eosinophils.

Thus, it is essential to study the quantitative and qualitative features of peripheral blood leukocytes in AB patients, particularly, of *in vivo* and *in vitro* lymphocytes of asthmatics.

MATERIALS AND METHODS

Study area: The study was carried out in Russia at the Kazan State University in the laboratory of the Department of Biochemistry of the Institute of Basic Medicine and Biology during the period from 12 June, 2013 to 20 September, 2019. The study's was part of the search for programmed death of lymphocytes in asthmatics according to the degree of severity.

Sampling and ethnic arrangements: The study was based on a selection of peripheral blood leukocytes from 30 Relatively Safe Donors (RSD) and patients with AB of variable severity; 69 of whom were males and 40 females with a mean age of 42+/-10 years. The patient's group is made up of patients of variable severity: 15 patients with intermittent asthma, 38 patients with mild persistent severity, 26 patients with moderate persistent severity and 30 patients with severe persistent asthma. The severity of asthma in these patients was assessed according to the global initiation for asthma guideline[16]. The diagnosis of AB was established based on the data of allergic anamnesis and based on the results of cutaneous experiments of skin with allergens and dust[17]. The work was performed following the rules of the ethics committee in the laboratory of clinical immunology and allergy of RKB and with the regulations of the Russian Federation Health Department in compliance with the Helsinki declaration.

Blood smear preparation: The blood smear was used to analyze figurative elements in blood, RSDs and AB patients. A drop of blood was placed on the surface of a slide 0.5-1 cm from one edge. With a rapid, gentle movement, the blood was spread over the entire surface of the slide using a polished coverslip narrow. After drying, the blood smear was immersed in undiluted fixative (eosin-methylene blue) (Abric+, Russia) for three min. After rinsing, the material was stained using the standard staining method recommended by Mai-Gron Valda Romanovsky and Gimz. The staining was done with the azure-eosin solution, diluted 10 times with distilled water for 15-20 min. After rinsing the well under a water pipe, air-dried the material and examined it with a binocular light microscope "Micro MC 50" (eyepiece 7 and objective-×100).

Leukocyte counts in RSD and AB patients of variable severity: The number of leukocytes was counted before and after erythrocyte bursting in 100 large tiles, using the Garaeva camera. About 20 microliters of venous blood stabilized by the anticoagulant EDTA was mixed with 0.4 mL of acetic acid and then stirred thoroughly. The garaeva camera was then filled with the prepared material and held for one minute in a horizontal position for settling of leukocytes. The number of leukocytes was counted in 100 large squares at low magnification (eyepiece ×10 and objective ×8) using the light microscope.

Isolation of lymphocytes: The zonal density gradient centrifugation method (1.077 g cm^{-1}) proposed by Patel *et al.*[18] was used, with slight modification. The 9 mL of

peripheral blood collected from the donors was transferred to a sterile tube containing heparin-Na in a ratio of 25 units per 1 mL of blood. The collected blood was then diluted with the previously prepared Xen kc solution to equal volume, i.e., 9 mL of Xen kc solution was added to 9 mL of blood and carefully and methodically stirred until a homogeneous blood solution was obtained. Carefully, 9 mL of the diluted blood was added to 9 mL of previously prepared ficoll-verograffin density gradient in a centrifuge tube. This was centrifuged for 40 min at 3000 rpm and 4°C. During centrifugation erythrocytes and granulocytes having a greater density than 1.077 g cm^{-1} are found at the bottom of the tube. On the surface of the density gradient, a white ring mostly of lymphocytes is formed. The lymphocyte suspension is transferred to a clean, sterile tube for washing. To the contents of the tube, 3-4 mL^{-1} of Xen kc solution were added and carefully stirred. The contents were then centrifuged for 10 min at 18°C and 2000 rpm (K24). After purification, the working solution consisted of 2×10^6 cells mL^{-1}.

Culture of lymphocytes: Lymphocytes isolated by the above method were diluted in RPMI 1640 solution such that in one well of the flat-bottom plate (Nung), 2×10^6 cells mL^{-1} is required. After dissolution, 10% calf embryo serum and 200 µg mL^{-1} L-glutamine was added (Flow)[19]. The cells were cultured in a 5% $C0_2$ incubator for 72 and 144 hrs.

Statistical analysis: The mathematical analysis was done on the staff computer with the use of the Excel program (Microsoft office 2003) and statistics 5.0. The comparison of the ranges of variations is made with the non-parametric Kryckaya-Yollica and Manna-Yitni T-criteria. The reliability of the difference in the frequency of the encountered indices was determined using the Fischer method and t-test and partly with the Bonferroni correction. The correctional analysis was done by the Rank and Cpirmena-rs method.

RESULTS

Particularities of the leukocyte cell count of DRS and AB patients *in vitro*: The study of the leukocyte content characteristics of peripheral blood shows that the leukocytes count in the blood of RSDs is 6.3×10^9 L^{-1} and that of asthmatic donors on average is 6.2×10^9. The total leukocyte count of asthmatic donors is slightly lower than that of RSDs. The number of lymphocytes decreases as asthma becomes more severe, from 37.80-17.30. Similarly, the eosinophil count decreases from 5.40 in mild asthmatics to 4.70 and 4.30 in moderate and severe asthmatics, respectively in Table 1.

The data in Fig. 1 presents the variation in the levels of the variable types of peripheral blood leukocytes in DRS in Fig. 1a and AB patients of different severity in Fig. 1b. The results showed a high level of neutrophils and eosinophils in asthmatics (Fig. 1b) compared to DRS (Fig. 1a). Neutrophils were significantly higher in DRS (59.76%) and asthmatics (63.22%).

The increase in neutrophils and eosinophils is offset by a decrease in lymphocytes and monocytes in Table 1 in AB patients.

The data in Fig. 2 present the variation of the content of figurative elements in peripheral blood of AB patients *in vivo*. It was observed a dependency relationship between the variation of lymphocytes in Fig. 2a, neutrophil in Fig. 2b, monocytes in Fig. 2c and eosinophils count in Fig. 2d and the degree of disease persistence. And this dependency relationship is statistically indisputable ($p \leq 0.05$).

Furthermore, regardless of severity, lymphocyte counts ranged from 2336.04×10^6-1069.14×10^6. Patients with mild disease had the highest lymphocyte counts (Fig. 2a). On the other hand, in terms of neutrophil populations, mild patients have the highest number of neutrophils (4585.56×10^6), while mild patients have the lowest number of neutrophils (Fig. 2b). In Fig. 2c, observed a variation in the monocyte count,

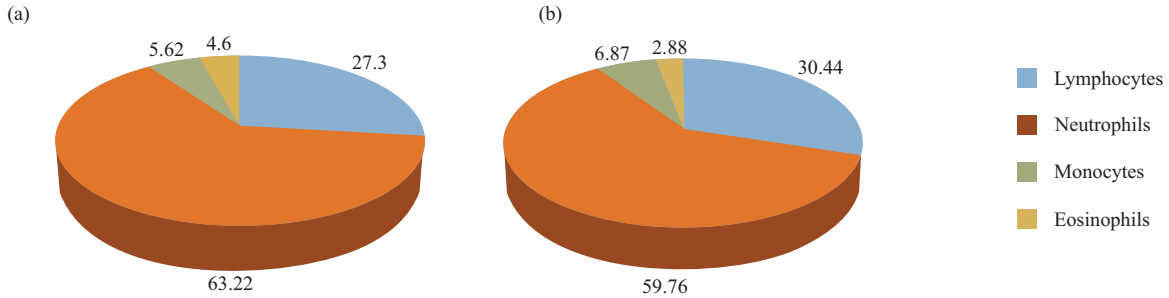

Fig. 1(a-b): Variation in the levels of the variable types of peripheral blood leukocytes in DRS and AB patients of different severity, (a) Variation in WBC count in DRS (%) and (b) Variation in WBC count in AB (%)

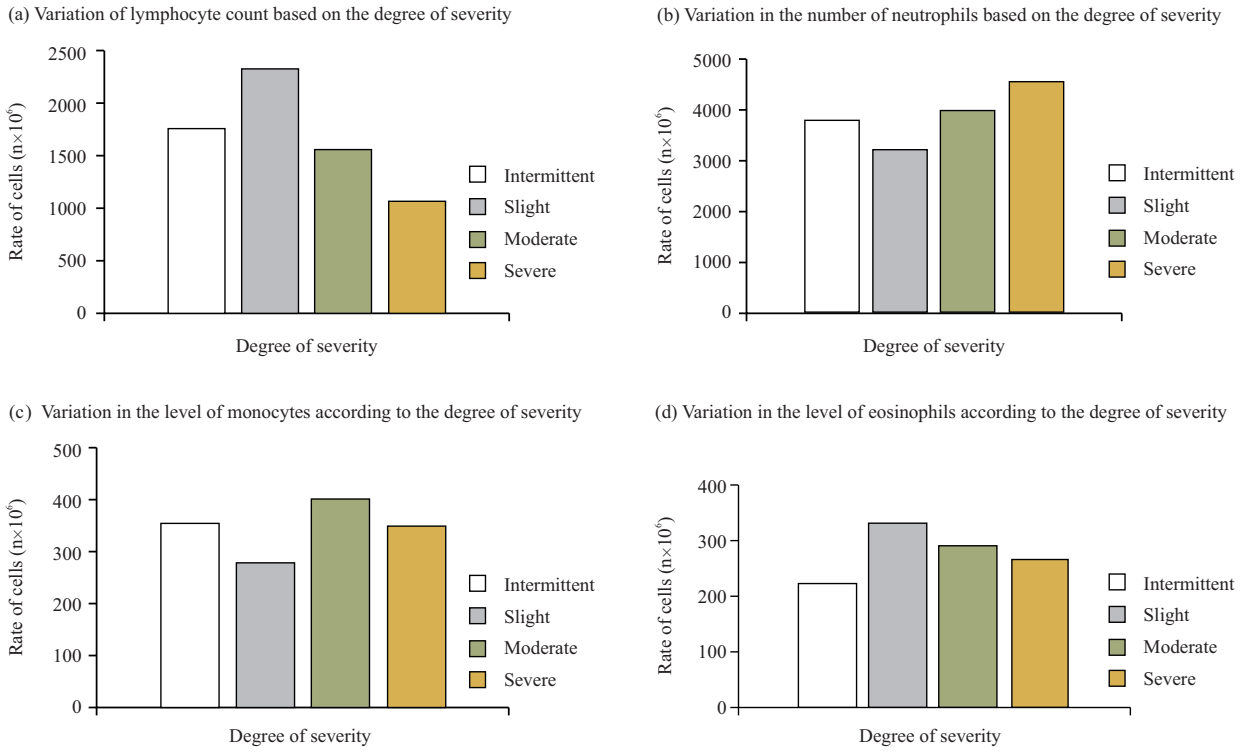

Fig. 2(a-d): Histogram of the variation of the content of figurative elements in peripheral blood of AB patients *in vivo*, (a) Lymphocytes count, (b) Neutrophils count, (c) Monocytes count and (d) Eosinophils count

Table 1: Variation of WBC count in RSD and AB patients depending on the degree of severity

Degree of asthma severity→	Intermittent (%)	Persistent (%)			AB 6.18×10^9 L^{-1}	N, (DRS) 6.3×10^9 L^{-1}		p-value
		Slight	Moderate	Severe	(%)			
Cells								
Lymphocytes	28.80	37.80	25.40	17.30	27.30	30.44	↓	0.05
Neutrophils	61.40	52.30	65.00	74.20	63.22	59.76	↑	0.04
Monocytes	5.80	4.50	6.50	5.70	5.62	6.87	↓	0.03
Eosinophils	3.65	5.40	4.70	4.30	4.60	2.88	↑	0.26

Table 2: Parameters of the mean values and standard errors of the sample data

Structural parameters: →	Number of patients (n, %)	X_{cp}	Σ
Degree of severity: ↓			
Intermittent	17 (25, 75)	1746.5	194.5
Persistent			
Slight	17 (25, 75)	1947.7	115.7
Moderate	15 (22, 75)	1837.8	153.3
Severe	17 (25, 75)	1588.7	138.2

depending on the severity level. Asthmatics of moderate severity have the highest monocyte count (400×10^6) while those of mild severity have the lowest. In Fig. 2d, see that the eosinophils count decreases with increasing asthma severity, with mild asthmatics having the highest eosinophil count, while severe asthmatics have the lowest.

Attention should be drawn to the behaviour of the lymphocyte count according to the degree of severity in Fig. 3. It can be observed that lymphocyte count decreases as the severity of the disease increases, which explains why patients with severe asthma have the lowest lymphocyte count.

The parameters of the mean value and standard error of the data for each group are shown in Table 2. There was a variation in the number of patients, depending on the degree of severity. There were 17 intermittent (25, 75%), 17 severe (25, 75%), 15 moderate (22, 75%) and 17 mild asthmatics (25, 75%).

Table 3: Median and percentile constant of the selected data

Structural parameters→	Number of patients (n, %)	Me	2.5-fold percentile	25th Perc.	75th Perc.	97.5th Perc.
Degree of severity: ↓						
Intermittent →	17 (25, 75)	1760	712.0	1161.0	1950.0	3508.8
Persistent						
Slight	17 (25, 75)	2005	1045.2	1695.0	2637.4	2244.0
Moderate	15 (22, 75)	1855	1039.4	1343.0	2092.5	2780.5
Severe	17 (25, 75)	1610	683.2	1088.0	1920.0	2503.2

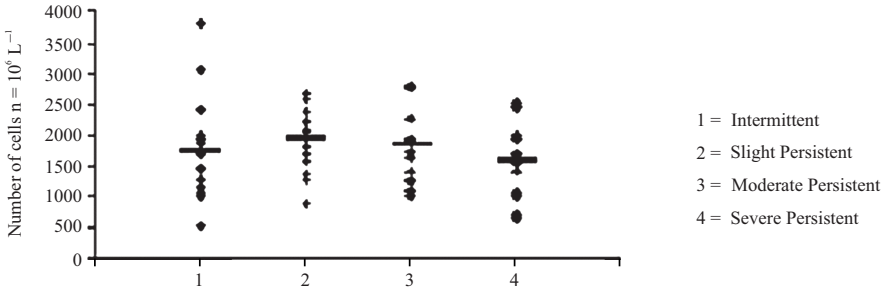

Fig. 3: Histogram of the distribution of lymphocyte count in AB patients according to the degree of severity
Mean value for each group is indicated on the histogram by the sign (--): 1-intermittent, 2-mild persistent, 3-moderate persistent and 4-severe persistent

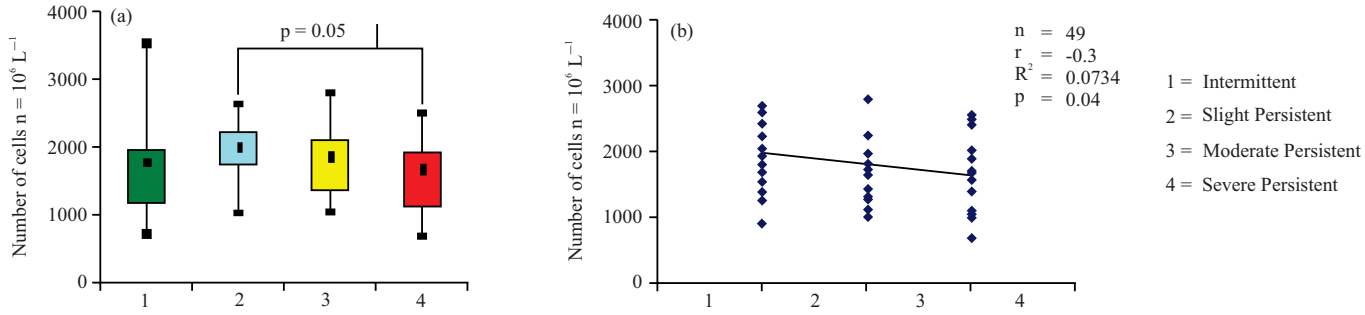

Fig. 4(a-b): Changes in lymphocyte count in intermittent and persistent asthma patients, (a) Intermittent and persistent asthma patients and (b) Only persistent asthma patients

The correlation of the lymphocyte count in peripheral blood depending on the degree of severity of the disease was then studied using the non-parametric criteria of Manna-Yitni corresponding to the structural unit (median and percentage). With the findings, it was deduced that the lymphocyte count constants in persistent asthma patients are characterized by a significant deviation (2.5 percentile = 712; 97.5 percentiles = 3508.8) (Table 3).

The progression from intermittent to mild persistent shows an increase in lymphocyte count (Me_{inter} = 1760; Me_{slight} = 2005) and the difference in lymphocyte count does not reach the level of certainty (p = 0.13), as distinct from the development of persistent severity disease where there is a decrease in lymphocyte count consistent with an increase in the degree of severity of the disease (Me_{slight} = 2005; Me_{mod} = 1850; Me_{severe} = 1610) in Table 3. These differences in lymphocyte count between mild and severe asthmatics are accurate to within 95% significance (p = 0.05) in Fig. 4a. The lymphocyte count in severe asthmatics is 30-50% lower than normal (17.3 vs. 24.-35% according to the WBC formula). Using the correlational analysis method, It is shown that in the persistent asthma groups, there is an inverse dependence between lymphocyte count and the degree of asthma severity (r = -0.3, p = 0.04) in Fig. 4b. Thus, the study of the total blood analysis constants of AB donors revealed that the leukocyte formula not only allows us to know which type of leukocytes is insufficient quantity but also to determine the degree of severity of the pathology.

There is a reduction in the lymphocyte count. This decrease may be related to the defective proliferative activation of lymphocytes and also to the migration of these cells from the peripheral blood to the lungs after allergen action. On the other hand, the prolongation of allergic inflammation during asthma is linked to the loss of their

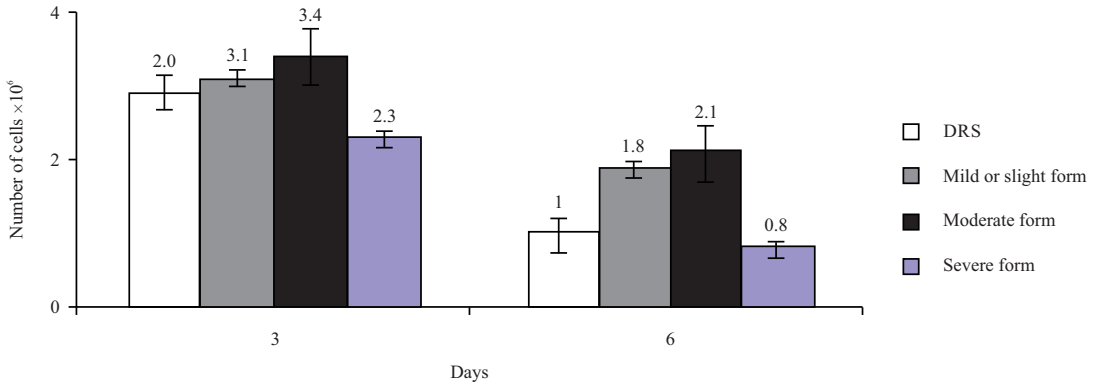

Fig. 5: Lymphocyte growth dynamics (%) of RSDs and asthmatics with mild, moderate-severe persistent severity during 72 and 144 hrs of the culture process

2 million lymphocytes-numbers of cells before culture marked by the black horizontal line

Table 4: Variation in cell count of DRS and persistent asthma patients *in vitro* (lymphocyte culture for 3 and 6 days)

	Bronchial asthma (degree of severity)							
	Cells culture (number, m mL^{-1})							
Days	DRS cells (control)	Change in number of cells, % (x)	Slight	Change in number of cells, % (x)	Moderate	Change in number of cells, % (x)	Severe	Change in number of cells, % (x)
0	2.0		2.0		2.0		2.0	
3	2.9	>de 45	3.1	>de 58	3.4	>de 70	2.3	>de 15
6	1.0	<de 65 (×3)	1.9	<38 (×1.6)	2.1	<37 (×1.7)	0.8	<65 (×2.8)

capacity to enter apoptosis. To explain the data obtained *in vivo*, the behaviour of asthmatic lymphocytes was studied according to their degree of severity *in vitro* by culturing them for 3-6 days in a standard culture medium.

Growth characteristics of lymphocytes in DRS and AB patients during their *in vitro* culture: The data in Table 4 showed the variation of the lymphocyte count *in vitro* during the culture process. After 3 days of culture, the lymphocyte count increased by 45% in DRS, 58-70% in mild and moderate asthmatics respectively. After 6 days of culture at the control level, the lymphocyte count decreased significantly by 3 times (i.e., by 65%) compared to that obtained in 3 days of culture. On the other hand, in mild and moderate asthmatics, the decrease is about 1.6-1.7 times, i.e., 37-38% compared to the number obtained after 3 days of culture. It can be concluded that there is a decrease in the death rate of lymphocytes of mild and moderate asthmatics during cell culture in Table 4.

Thus, the lymphocyte count varies with increasing time in both the DR's lymphocyte culture and AB patients in Fig. 5.

In the culture medium, lymphocytes from mild and moderate asthmatics continued to multiply but their proliferative activity was some what decreased (the number of cells increased by 20-33%, respectively), which could explain the death of cells. After 6 days of culture (Table 4), a decrease in the number of cells in the culture medium of DRS and AB patients was noticed. Therefore, *in vitro* lymphocytes number varied in the culture time in both DRS and AB donors, suggesting variable degrees of cell survival according to the disease severity. The peculiarities of lymphocyte growth *in vitro* were established, reflecting the difference in lymphocyte proliferative activity. According to culture data, lymphocytes of asthmatics are characterized by a slowing down of apoptosis, on the one hand, expressed by a prolongation of cell survival. On the other hand, with the data obtained, the number of lymphocytes of severe asthmatics is undoubtedly lower than in DRS. Furthermore, lymphocytes from mild and moderate asthmatics *in vitro* show a lymphoproliferative activity (lymphocytosis) expressed by an increase in the number of cells in the blood of mild and moderate asthmatics.

DISCUSSION

Despite the advanced studies in the field of immunology, the functional diagnosis of AB remains a current problem in terms of the untimely organization that fails therapy[20,21]. The multiple types of research concerning the study of the different drug mean namely the variable forms of drug

combinations for the treatment of diseases and the dosage regime can contribute to the improvement of the control of the disease course[22]. The result of a lack of ultrasensitive and universal data on asthma control has led to the creation of several control assessment systems based on clinic-functional data analysis. In the daily practice of clinicians, the level of control of asthma severity is relatively low. Even in Western European countries with a highly developed public health system, this rate is around 5%. In Russia, the golden rule on asthma symptom control reached 6.6% for patients receiving basic therapy and 3.3% for those using only symptomatic therapy[23]. Perhaps ultimately, this is related to a high level of knowledge of cellular and molecular biochemistry and the basis of the genesis of this disease. Therefore, the search for new cellular markers may allow the diagnosis of the early stages of chronic obstructive lung disease in general and asthma in particular at the cellular and molecular levels[24,25].

Some scientific evidences[26] have highlighted the role of apoptosis and atopy in the pathogenesis of chronic airway diseases and particular asthma. The role of the cell death mechanism in allergy is being actively studied. The particular interest is conditioned by the fact that based on allergic diseases is observed the activation of immunocompetent cells and accumulation of self-reactive clones[27] in the bronchi and the loss of epithelial cells at the level of bronchitis[28,29]. It is assumed that the programmed death of the majority of T-lymphocytes is related to their migration following the action of antigens, i.e., T-cell apoptosis is considered by some authors as a mechanism of antigen-directed lymphocyte selection[30]. In asthma, airway inflammation is characterized by recruitment and retention of immunocompetent cells[31,32] in the bronchi and sometimes to their resistance to cell death by apoptosis[33]. The most important in this field of research is the work of Vignola[34], who with the TUNEL method demonstrated apoptosis of eosinophils, macrophages and lymphocytes in the tissues of the arterial trachea of healthy individuals and individuals with chronic bronchitis. The opposite process to apoptosis is cell multiplication[35]. At the cellular scale, the maintenance of the biological integrity of the human organism is achieved by a regulatory influence of afferent signals that support the state of equilibrium between the 3 integrated physiological processes: Proliferation, differentiation and programmed cell death. The experimental model for the study of asthma in mice using the immunofluorescent cell labelling method showed migration of leukocytes from the peripheral blood to the lungs for 22 hrs after the allergens action[36]. This indicates that the study of peripheral blood leukocytes can serve as a model for the state of the immune response in tissues. In the clinic, it is not only the total number of leukocytes in the important blood but also the ratio of all leukocyte cells. Changes in the WBC count are associated with several diseases that are non-specific. Nevertheless, it gives the idea of the health status of the patient and the effectiveness of the treatment procedure. From the literature data, in RSD the number of leukocytes in 1 L of blood is 6.0-9.0.10^9[37]. In asthmatics, the overall leukocyte count in the blood differs slightly from the leukocyte count in the blood of RSDs.

However, a detailed study of the different types of leukocytes in the blood shows a definite increase in the number of neutrophils ($p = 0.04$) and eosinophils ($p = 0.26$) compared to normal. It should be noted that the increase in neutrophils and eosinophils is compensated by a decrease in lymphocytes and monocytes. It could be assumed that the variable types of leukocytes play a major role in the evolution of asthmatic inflammation. The most recent work has shown the existence of Innate Lymphoid Cells (ILCs) which are involved in various inflammatory conditions such as asthma. The interaction of ILCs with other types of leukocytes is mediated by the expression on the surface of ILCs of ligand (ICOSL) and specific receptor (ICOS) known for their role in the survival and proliferation of T-cell subpopulations[38]. ILCs appear to play a crucial role both directly and indirectly in the initiation and direction of the inflammatory response. These data show that lymphocytes play an important role in bronchial asthma and make these cells a relevant target in this pathology[10]. Particularly, the dynamics of decreasing lymphocyte numbers following the degree of disease severity were observed. The use of the non-parametric Manna-Yitni T-criteria method showed that differences at the peripheral blood level between the number of lymphocytes of patients of mild and severe severity persisting during Asthma are authentic up to 95% ($r = 0.05$). The correlational analysis method revealed an inverse dependency between lymphocyte count and the disease severity ($r = -0.3$, $p = 0.04$, $R_2 = 0.0734$, $n = 49$). The decrease in lymphocyte number may be related to a disruption of lymphocyte proliferative activity and their migration from the peripheral blood to the lungs under the influence of allergens. To explain the results obtained *in vivo*, cells behaviour *in vitro* was studied. In most culture systems, lymphocytes perish by apoptosis after a few days. We found that the number of lymphocytes obtained in 3 days increased by 45% in the cell culture of healthy donors and by 58-70% for mild and moderate asthmatics respectively. Findings allow us to conclude that *in vitro*, the lymphocyte rate changes according to the culture time in both healthy and sick individuals. This helps conclude that the survival of the cells varies according to the degree of severity. But on one

hand, the results of our studies show an inverse relationship between the number of peripheral blood lymphocytes and the degree of asthma severity *in vivo*. On the other hand, based on the slowing down of cell development in the culture medium of severe patients, lymphocytes from mild and moderate asthmatics showed a proliferative activity (lymphocytosis).

Regarding the obtained results, it can be said that quantitative estimation of certain elements of peripheral blood of AB patients is of interest in the sense that it would make it possible to know the level of evolution of the disease in patients, the effectiveness of the administered treatment and the adaptation of the treatment according to the disease progression in a patient.

CONCLUSION

The investigation on the leukocyte formula of the variable asthma groups has shown a significant difference in the rate of the different types of leukocytes in the blood. This difference increases with the degree of severity of the disease and may be the consequence of the initiation of an immune-inflammatory response.

SIGNIFICANCE STATEMENT

This study allowed us to quantify certain leukocyte cells and to demonstrate the existence of a dependent relationship between the level of lymphocytes and the degree of severity of asthma. The results obtained could be beneficial for researchers in the search for predictive cell markers and would help health workers to better understand the etiopathology of the disease for early diagnosis. The data suggest that lymphocytes also play a major role in the etiopathology of asthma.

REFERENCES

1. Abdulamir, A.S., R.R. Hafidh, F. Abubakar and K.A. Abbas, 2008. Changing survival, memory cell compartment and T-helper balance of lymphocytes between severe and mild asthma. BMC Immunol., Vol. 9. 10.1186/1471-2172-9-73.
2. Schleich, F. and R. Louis, 2012. Targeted asthma therapies: Confirmations, hopes and disappointments. Rev. Med. Liège, 67: 14-21.
3. Holgate, S.T., D.E. Davies, P.M. Lackie, S.J. Wilson, S.M. Puddicombe and J.L. Lordan, 2000. Epithelial-mesenchymal interactions in the pathogenesis of asthma. J. Allergy Clin. Immunol., 105: 193-204.
4. Spertini, F., 2001. Asthma immunotherapy: New specific antagonists. Rev. Med. Suisse, 59: 15-17.
5. Fievez, L., G. Seumois, P. Lekeux and F. Bureau, 2005. Neutrophil apoptosis. Ann. Med. Vet., 149: 10-19.
6. Lambrecht, B.N. and H. Hammad, 2015. The immunology of asthma. Nat. Immunol., 16: 45-56.
7. Brusselle, G.G., T. Maes and K.R. Bracke, 2013. Eosinophils in the spotlight: Eosinophilic airway inflammation in nonallergic asthma. Nat. Med., 19: 977-979.
8. Holgate, S.T. and P.D. Sly, 2020. Asthma Pathogenesis. In: Middleton's Allergy: Principles and Practice, Burks, A.W., S.T. Holgate, R.E. O'Hehir, L.B. Bacharier, D.H. Broide, G.K. Hershey and R.S. Peebles (Eds.)., Elsevier Academic Press, United States, ISBN-13: 9780323544245, .
9. Crinier, A., C. Viant, M. Girard-Madoux and É. Vivier, 2017. Innate lymphoid cells. Méd. Sci., 33: 534-542.
10. Zou, X.L., Z.G. Chen, T.T. Zhang, D.Y. Feng, H.T. Li and H.L. Yang, 2018. Th17/Treg homeostasis, but not Th1/Th2 homeostasis, is implicated in exacerbation of human bronchial asthma. Ther. Clin. Risk Manage., 14: 1627-1636.
11. Klein, M., E. Dijoux, F. Dilasser, D. Hassoun and A. Moui *et al*., 2019. New protagonists in asthma pathophysiology. La Presse Médicale, 48: 255-261.
12. Neill, D.R., S.H. Wong, A. Bellosi, R.J. Flynn and M. Daly *et al*., 2010. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. Nature, 464: 1367-1370.
13. Laffont, S., E. Blanquart and J.C. Guéry, 2018. Sex-bias in allergic asthma: Androgens and group 2 innate lymphoid cells. Med. Sci., 34: 247-252.
14. Kumar, R.K. and P.K. Jeffery, 2020. Pathology of Asthma. In: Middleton's Allergy: Principles and Practice, Burks, A.W., S.T. Holgate, R.E. O'Hehir, L.B. Bacharier, D.H. Broide, G.K. Hershey and R.S. Peebles (Eds.)., Elsevier Academic Press, United States, ISBN-13: 9780323544245, .
15. Lavinskiene, S., K. Malakauskas, J. Jeroch, D. Hoppenot and R. Sakalauskas, 2015. Functional activity of peripheral blood eosinophils in allergen-induced late-phase airway inflammation in asthma patients. J. Inflammation, Vol. 12. 10.1186/s12950-015-0065-4.
16. Bateman, E.D., S.S. Hurd, P.J. Barnes, J. Bousquet and J.M. Drazen *et al*., 2008. Global strategy for asthma management and prevention: Gina executive summary. Eur. Respir. J., 31: 143-178.
17. White, J., J.Y. Paton, R. Niven and H. Pinnock, 2018. Guidelines for the diagnosis and management of asthma: A look at the key differences between BTS/SIGN and NICE. Thorax, 73: 293-297.
18. Patel, D., C.P. Rubbi a nd D. Rickwood, 1995. Separation of T and B lymphocytes from human peripheral blood mononuclear cells using density perturbation methods. Clin. Chim. Acta, 240: 187-193.

19. Lehmann, J.S., P. Rughwani, M. Kolenovic, S. Ji and B. Sun, 2019. LEGENDplex™: Bead-Assisted Multiplex Cytokine Profiling by Fow Cytometry. In: Methods in Enzymology. Galluzzi, L. and N.P. Rudqvist (Eds.)., Elsevier, Amsterdam, Netherlands, pp: 151-176.
20. Alode, V.C.J., B.M. Lamine, P. Ylia, S. Alphonse and A.Z. Ivanovna, 2011. The implication of morphological characteristics in the etiology of allergic asthma disease and in determining the degree of severity of atopic and bronchial asthma. Asian J. Cell Biol., 6: 65-80.
21. Agarwal, R., S. Dhooria, A.N. Aggarwal, V.N. Maturu and I.S. Sehgal *et al*., 2015. Guidelines for diagnosis and management of bronchial asthma: Joint ICS/NCCP (I) recommendations. Lung India, 32: S3-S42.
22. Barnes, P.J., 2004. New drugs for asthma. Nat. Rev. Drug Discovery, 3: 831-844.
23. Lenskaya, L.G., L.M. Ogorodova, M.V. Malakhovskaya and O.S. Kobyakova, 2004. Analysis of direct medical costs for the treatment of bronchial asthma in the tomst region. Pulmonology, 4: 37-43.
24. Nadif, R., V. Siroux, M.P. Oryszczyn, C. Ravault, C. Pison, I. Pin and F. Kauffmann, 2009. Heterogeneity of asthma according to blood inflammatory patterns. Thorax, 64: 374-380.
25. Siroux, V., X. Basagana, A. Boudier, I. Pin and J. Garcia-Aymerich *et al*., 2011. Identifying adult asthma phenotypes using a clustering approach. Eur. Respir. J., 38: 310-317.
26. Urbonien, D., R. Skalauskas and B. Sitkauskiene, 2005. Autoimmunity in pathogenesis of chronic obstructive pulmonary disease. Medicina (Kaunas), 41: 190-195.
27. Chang, P.J., P.K. Bhavsar, C. Michaeloudes, N. Khorasani and K.F. Chung, 2012. Corticosteroid insensitivity of chemokine expression in airway smooth muscle of patients with severe asthma. J. Allergy Clin. Immunol., 130: 877-885.E5.
28. Trautmann, A., M. Akdis, K. Blaser and C.A. Akdis, 2000. Role of dysregulated apoptosis in atopic dermatitis. Apoptosis, 5: 425-429.
29. Halwani, R., A. Sultana, A. Vazquez-Tello, A. Jamhawi, A.A. Al-Masri and S. Al-Muhsen, 2017. Th-17 regulatory cytokines IL-21, IL-23 and IL-6 enhance neutrophil production of IL-17 cytokines during asthma. J. Asthma, 54: 893-904.
30. Rottem, M. and Y. Shoenfeld, 2003. Asthma as a paradigm for autoimmune disease. Int. Arch. Allergy Immunol., 132: 210-214.
31. Jones, C.P., L.G. Gregory, B. Causton, G.A. Campbell and C.M. Lloyd, 2012. Activin a and TGF-β promote T_H9 cell-mediated pulmonary allergic pathology. J. Allergy Clin. Immunol., 129: 1000-1010.E3.
32. Kerzerho, J., H. Maazi, A.O. Speak, N. Szely and V. Lombardi *et al*., 2013. Programmed cell death ligand 2 regulates T_H9 differentiation and induction of chronic airway hyperreactivity. J. Allergy Clin. Immunol., 131: 1048-1057.E2.
33. Alode, V.C.J., B.M. Lamine, P. Ylia, S. Alphonse and A.Z. Ivanovna, 2011. The implication of morphological characteristics in the etiology of allergic asthma disease and in determining the degree of severity of atopic and bronchial asthma. Asian J. Cell Biol., 6: 65-80.
34. Vignola, A.M., G. Chiappara, R. Gagliardo, M. Gjomarkaj and A. Merendino *et al*., 2000. Apoptosis and airway inflammation in asthma. Apoptosis, 5: 473-485.
35. Jan, R. and G.E.S. Chaudhry, 2019. Understanding apoptosis and apoptotic pathways targeted cancer therapeutics. Adv. Pharm. Bull., 9: 205-218.
36. Schuster, M., T. Tschernig, N. Krug and R. Pabst, 2000. Lymphocytes migrate from the blood into the bronchoalveolar lavage and lung parenchyma in the asthma model of the brown Norway rat. Am. J. Respir. Crit. Care Med., 161: 558-566.
37. Barnig, C., A. Purohit, A. Casset, C. Sohy, F. Lieutier-Colas, E. Sauleau and F. de Blay, 2013. Nonallergic airway hyperresponsiveness and allergen-specific IgE levels are the main determinants of the early and late asthmatic response to allergen. J. Invest. Allergol. Clin. Immunol., 23: 267-274.
38. Maazi, H., N. Patel, I. Sankaranarayanan, Y. Suzuki and D. Rigas *et al*., 2015. ICOS:ICOS-lig and interaction is required for type 2 innate lymphoid cell function, homeostasis and induction of airway hyperreactivity. Immunity, 42: 538-551.