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Seasonal variations in the haematological parameters RBC, RBC indexes, Hgb and Plt count in the Bulgarian population

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Abstract

Background: Sources of variation in laboratory results can be pre-analytical, analytical and biological. Biological sources of variation are often used as factors for stratifying reference intervals. The aim of the study is to investigate the seasonal variation in the haematological parameters red blood cells (RBCs), RBC indexes (MCV – mean cell volume; MCH mean cell hemoglobin and MCHC - mean hemoglobin concentration in erythrocytes), haemoglobin (Hgb) and platelet counts (Plts), within reference limits, in the Bulgarian population.

Material and methods: The research was carried out in UH St. Ivan Rilski, Sofia, for one year period (January - December 2022). Results outside the reference intervals are excluded. The following parameters are evaluated: RBCs, RBC indexes and Hgb in 13,197 men and 18,729 women, and Plts in 18,125 men and 21,018 women. Alinity hq, Abbott haematological analyser is used.

Results: RBC and Hgb levels are higher in males (+9% and 11%, respectively). In women, a higher level of Plts is found with approximately 5%. It is observed a winter (November and December) decrease in RBC (in men - 5.8% and in women - 4.6%) and an increase in Plts in December - in women with + 4.2% and in men with + 3% compared to summer. Hgb increases slightly from August to December +2.7% for men and women. Gradually from September to December, MCH, MCHC and MCV are increasing in both sexes.

Conclusion: The changes in the studied hematological indicators provide an evidence that these fluctuations are not random but related to the seasons. Sex and age differences are also observed in the present study with no alteration in the seasonal variation.

Key words: seasonal variation, biological rhythm, reference limits, platelets, erythrocytes

Introduction

Humanity has always been interested in the rhythm of life - through empiricism and the occult, to the most modern scientific techniques - from the rhythm of the ladybird to the cosmic rhythm. Life is an ocean of vibrations and rhythm is fundamental to our lives. Earth's circadian, seasonal, other cycles and resonant electromagnetic oscillations are a symphony of rhythms in which life exists (1).

Biological variation is defined as the normal physiological fluctuation of biomarkers around a stable homeostatic point. Their application in clinical practice brings patient care closer to personalized medicine. After all, the influence of biological variation is an important factor in the establishment of reference limits and medical decision-making. There are various biological variations - some with short-term effects, others with longer-term effects and also permanent (2).

Most often, medical attention to annual rhythms is primarily directed at inflammation and immune competence. Our previous observations in the study, carried out in the city of Sofia - Bulgaria, reveal seasonal fluctuations of the white blood cell counts (3).

The aim of the study is to investigate the seasonal variation in the hematological parameters red blood cells (RBCs), RBC indexes (MCV – mean cell volume; MCH mean cell hemoglobin and MCHC - mean hemoglobin concentration in erythrocytes), hemoglobin (Hgb) and platelet counts (Plts), within reference limits, in the Bulgarian population

Material and methods

The study is carried out during January 1 to December 31, 2022 at the University Hospital St. Ivan Rilski, Sofia, Bulgaria. The total number of samples is 150,000, including outpatients and patients hospitalized in Clinics and Departments of the Hospital - Gastroenterology, Nephrology, Pulmonology, Neurology, Abdominal Surgery, Neurosurgery, Hematology and Oncology. As expected, most of the results are pathologic.

Criteria for the result selection are as follows: only results, within the reference intervals, for adults over 18 years old are included; abnormal Hgb levels and Plts are excluded. The distribution of the tested samples comprises 13,197 male samples and 18,729 females for RBCs and 18,125 males and 21,018 females for Plts. The distribution of the included groups is presented in Table 1.

Table 1. Distribution of all included samples in different groups according the sex and age. Only individuals with results in reference ranges were included.

	Individuals examined for Red blood cells		**Individuals examined for Platelets**	
Age (years)	**women**	**men**	**women**	**men**
18-35	1 466	1 531	1 520	1 540
36-65	10 472	7 930	10 895	9 659
>65	6 791	3 736	8 603	6 926
total	18 729	13 197	21 018	18 125

Complete blood count is examined in whole blood specimen. The blood is collected into tubes for hematology testing with K_2EDTA (dipotassium ethylenediaminetetraacetic acid) as anticoagulant. Sampling is done in compliance with the aseptic procedures and according to the recommendations for standardisation in the pre-analytical phase of the laboratory testing process. The samples are analysed up to 30 min after the blood collection by hematology analyser Alinity hq, Abbott.

Results

Red blood cells

The results of RBCs, MCV, MCH, MCHC and Hgb are presented in Table 2 for men and Table 3 for women as mean values ± SD.

Table 2. Results for RBC, erythrocytes indexes (MCV, MCH and MCHC) and Hgb in men

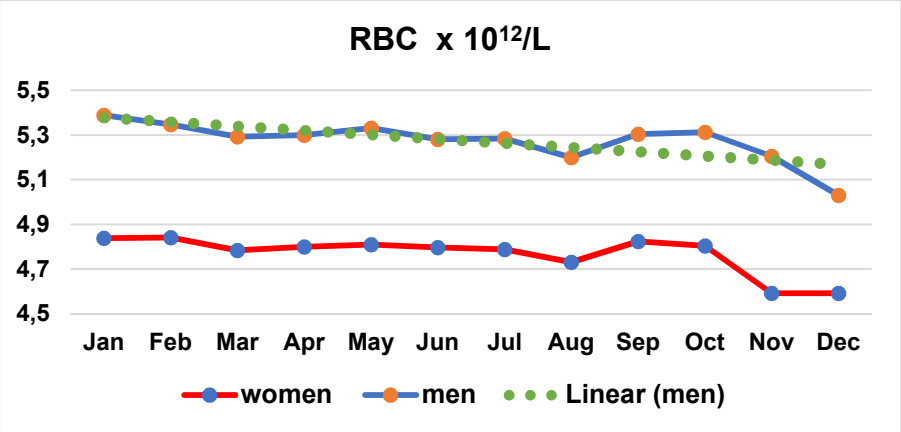
ID	n	Age (years)	RBCx10^{12}/L	Hb g/L	MCV fL	MCH pg	MCHC g/L
Jan	1001	53.8±9.9	5.4±0.5	152.0± 9.5	84.5 ±5.3	28.4 ±2.1	335.8±18.7
Feb	1089	52.8±15.6	5.3 ±0,5	151.3 ±9.1	84.3 ±4,5	28.4 ±1.8	337.6±18.3
Mar	1094	53.8±15.5	5.3 ±0.5	151.7 ±9.7	84.5 ±5.2	28.8 ±2.0	341.3±20.9
Apr	953	54.0±14.8	5.3±0.5	152.9±9.5	84.8±5.2	29.0±2.2	342.6±24.6
May	1203	53.6±14.5	5.3 ±0.5	152.8 ±9.7	85.1 ±4.8	28.8 ±1.9	338.4±16.9
Jun	1713	54.5±14.9	5.3 ±0.5	151.5 ±9.8	85.1 ±4.7	28.8 ±1.8	338.6±17.0
Jul	938	54.0±15.2	5.3 ±0.5	151.9 ±9.4	84.8 ±4.4	28.9 ±1.9	340.5±16.5
Aug	1024	54.0±14.8	5.2 ±0.5	150.3 ±9.2	84.6 ±5.6	29.1 ±2.5	343.7±18.5
Sep	1104	51.8±15.0	5.3 ±0.5	151.9±9.8	85.3 ±5.2	28.8 ±2.0	337.6±15.0
Oct	1107	52.5±15.2	5.3±0.5	152.4±9.6	84.9±4.9	28.8±2.0	340.1±20.3
Nov	1012	54.5±15.0	5.2±0.5	154.1±9.5	85.2±4.9	29.7±2.1	349.5±17.0
Dec	959	53.3±15.5	5.0±0.5	154.4±10.4	87.5±5.2	30.8±2.0	352.4±12.7

Table 3. Results for RBC, erythrocytes indexes (MCV, MCH and MCHC) and Hgb in women

ID	Women	Age years	RBCx10^{12}	Hb g/L	MCV fL	MCH pg	MCHC g/L
Jan	1379	57.4±14.3	4.8±0.4	136.2±8.7	85.3±4.9	28.3±2.1	331.8±16.4
Feb	1430	56.1±14.9	4.8±0.4	135.7±8.6	84.8±4.7	28.2±2.0	332.0±16.9
Mar	1564	58.1±14.4	4.8±0.4	135.9±8.8	84.9±4.7	28.6±2.3	336.5±21.0
Apr	1322	58.5±14.7	4.8±0.4	136.6±9.1	85.1±4.6	28.6±2.1	336.1±19.7
May	1660	59.0±14.3	4.8±0.4	136.2±9.2	85.2±4.6	28.4±2.0	333.8±14.3
Jun	1825	58.5±14.3	4.8±0.4	136.1±8.7	85.2±4.7	28.5±1.9	334.4±14.0
Jul	1435	57.7±14.9	4.8±0.4	136.1±8.9	84.7±4.6	28.5±2.0	337.1±14.8
Aug	1490	57.9±14.5	4.7±0.4	134.9±8.4	84.6±4.7	28.6±2.1	338.6±16.1
Sep	1793	56.5±14.1	4.8±0.4	136.0±8.8	85.0±5.0	28.3±2.0	333.1±13.6
Oct	1801	57.3±14.4	4.8±0.4	136.3±9.1	84.7±4.6	28.5±2.0	336.4±18.0
Nov	1930	58.6±13.5	4.6±0.4	137.1±8.8	84.0±5.5	28.9±2.4	344.2±15.8
Dec	1100	58.6±15.4	4.6±0.4	138.6±9.7	86.8±5.1	30.3±2.2	348.8±13.4

RBCs are higher in males with approximately 9%. Winter (November and December) decline in the number of erythrocytes is observed: - 5.8% in men and - 4.6% in women, compared to the autumn. Decrease in the number of erythrocytes is observed in August for both sexes (Fig. 1).

Figure 1. Annual variations of red blood cells, according to the sex



In men, the Hgb is higher by 12%. For both sexes, it is 1% lower in August compared to adjacent months. Increase in Hgb from August to December + 2.7% for both men and women is observed. Gradually, during the period September - December 2022, MCHC (+4%) and

MCH (about +7%) increase. In both sexes, MCV is sharply increased in December with 2.7%, as seen in the variation in Hgb (Fig. 2 – 5).

Figure 2. Annual variations of hemoglobin, according to the sex

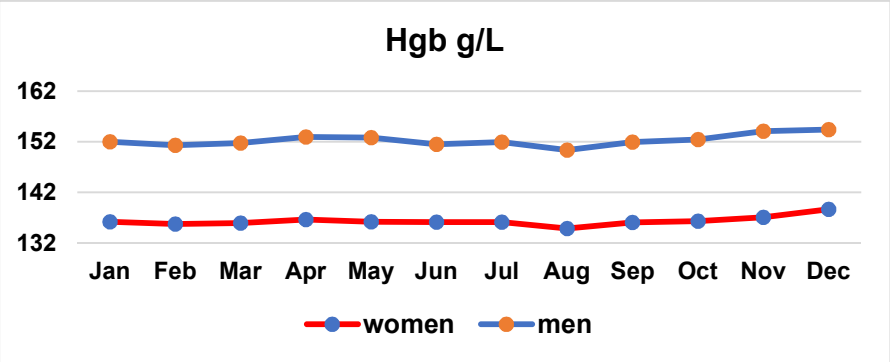


Figure 3. Annual variations of MCV, according to the sex

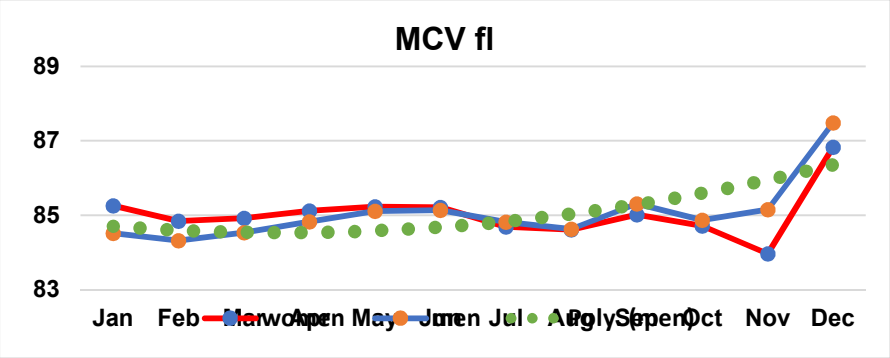


Figure 4. Annual variations of MCH, according to the sex

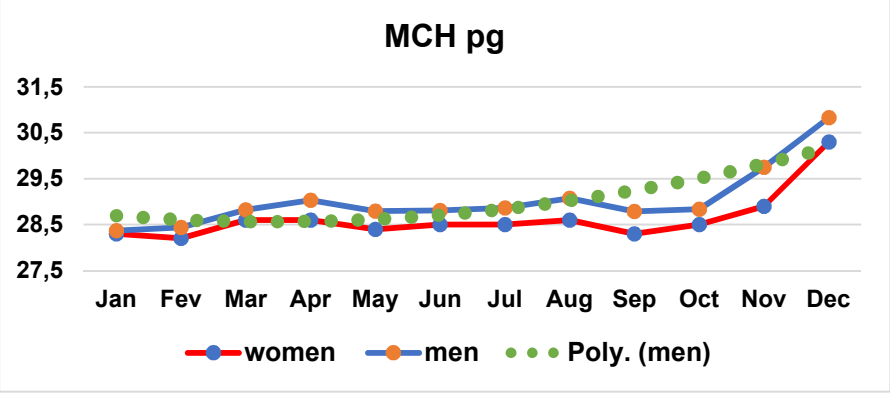
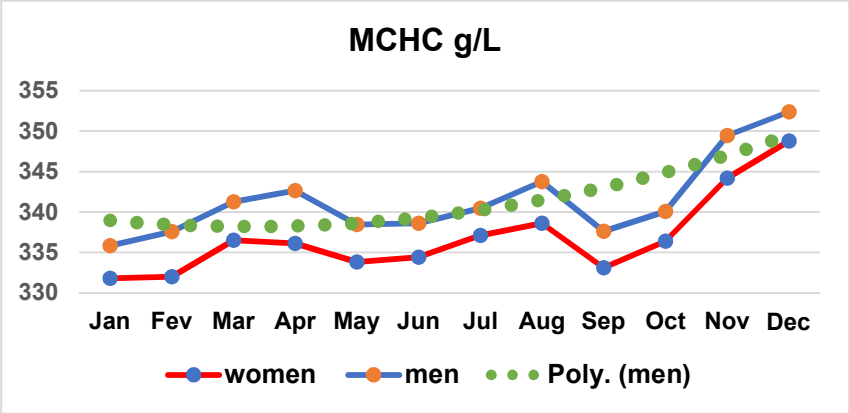


Figure 5. Annual variations of MCHC, according to the sex



One of the most strongly influencing factors of biological variation in medicine, especially on the results of a complete blood count is the age. It affects all cells. The results of the present study are presented in Figures 6 - 10. Seasonal fluctuations are noticed in both main age groups independent of sex - over 65 - 8.8% for RBC and -7% for Hgb. There is no sex difference in index fluctuations.

Figure 6. Annual variations of RBC, according to the age

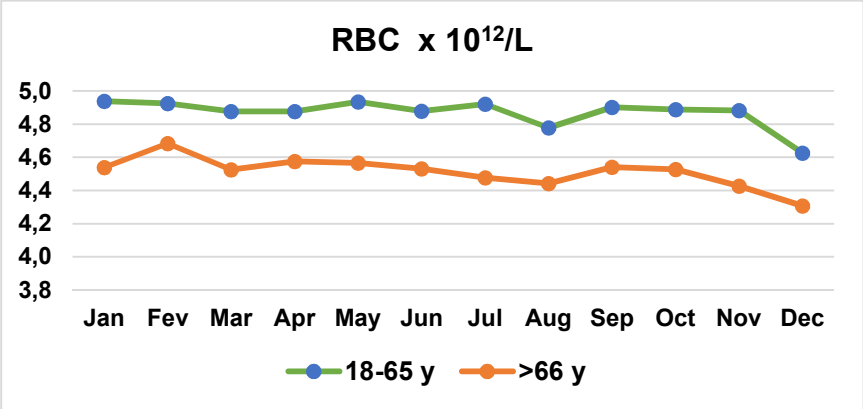


Figure 7. Annual variations of Hgb, according to the age

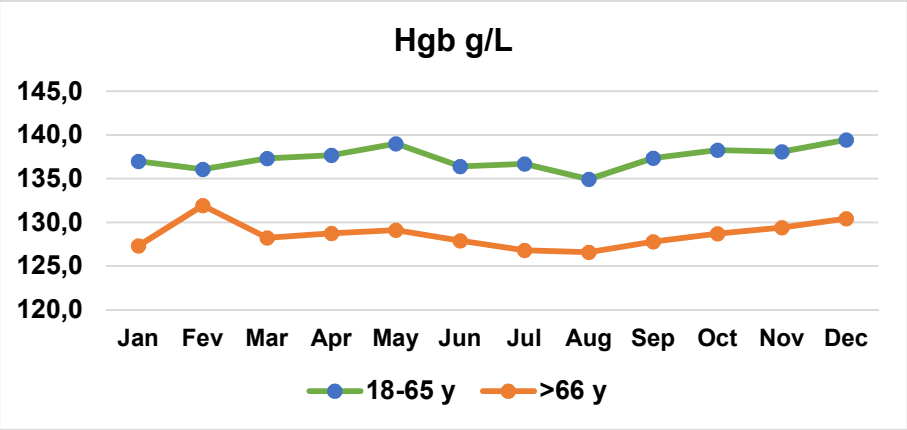


Figure 8. Annual variations of MCV, according to the age

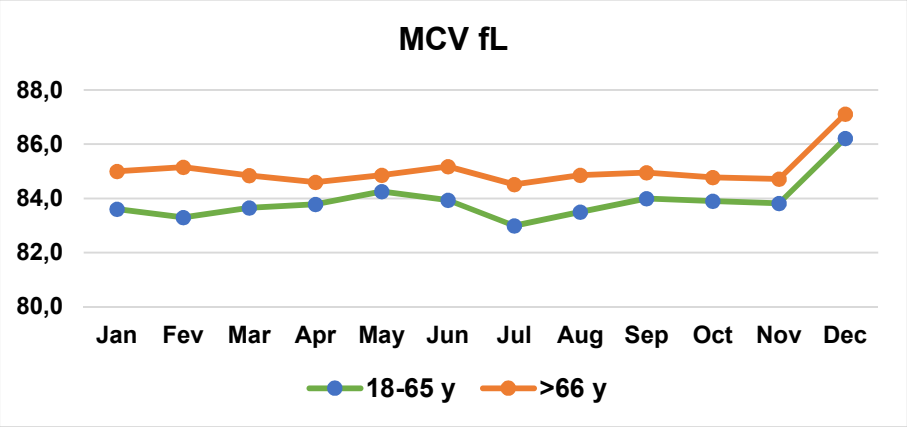


Figure 9. Annual variations of MCH, according to the age

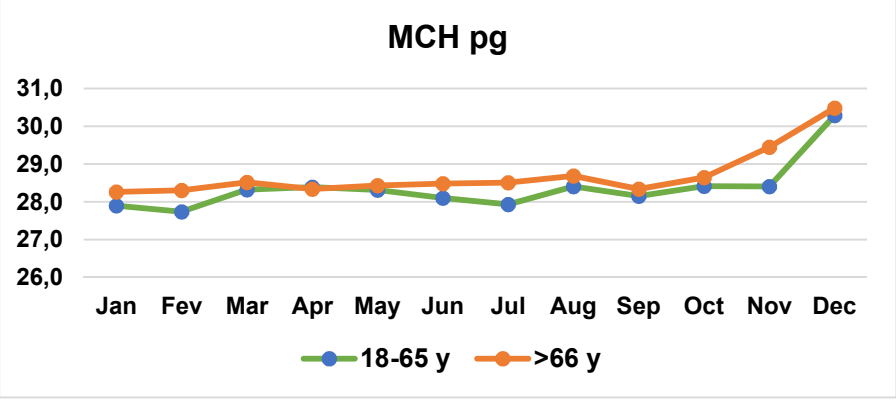
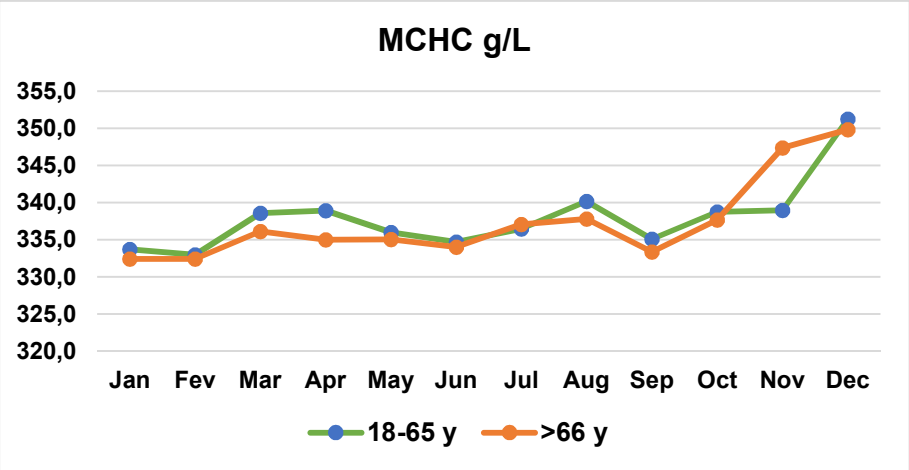


Figure 10. Annual variations of MCHC, according to the age



The age dependence of erythrocyte's number, Hgb and their relations, in both sexes, with increasing age, is presented in Tables 4 and 5 and Fig. 11 - 15.

Table 5. Results (mean values ± SD) within reference range, distributed according to the age, in men

Age; years	n	RBC x10^{12}/L	Hgb g/L	MCV fL	MCH pg	MCHC g/L
18-35	1531	5.5±0.5	154.0±9.8	82.9±5.3	28.4±2.2	342.7±19.4
36-65	7930	5.2±0.5	152.0±9.9	85.1±5.1	29.1±2.2	342.7±19.5
>65	3736	5.1±0.6	148.0±9.6	86.1±5.4	29.3±2.5	340.6±19.5

Table 6. Results (mean values ± SD) within reference range, distributed according to the age, in women

Age; years	n	RBCx10^{12}/L	Hgb g/L	MCV fL	MCH pg	MCHC g/L
18-35	1466	4.8±0.4	134.5±8.3	83.4±4.5	28.2±2.0	338.6±17.9
36-65	10472	4.8±0.5	136.1±9.2	85.1±5.2	28.8±2.3	338.1±17.8
>65	6791	4.7±0.5	134.7±9.3	85.4±5.4	28.9±2.8	338.2±21.4

The mean value of RBC results within the reference interval is decreased with age in men (-7.8%), also of Hgb (-4%) but the same test results are not changed in women. MCV and MCH are increased (+2 -- + 4%) for both sexes. MCHC falls below 1%.

Figure 11. Red blood cell results (within the reference limits) according to the sex and age

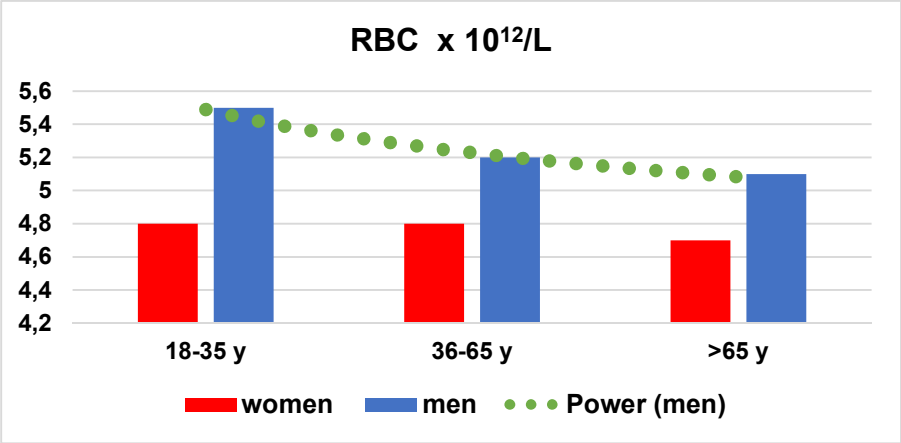


Figure 12. Hemoglobin (within the reference limits) according to the sex and age

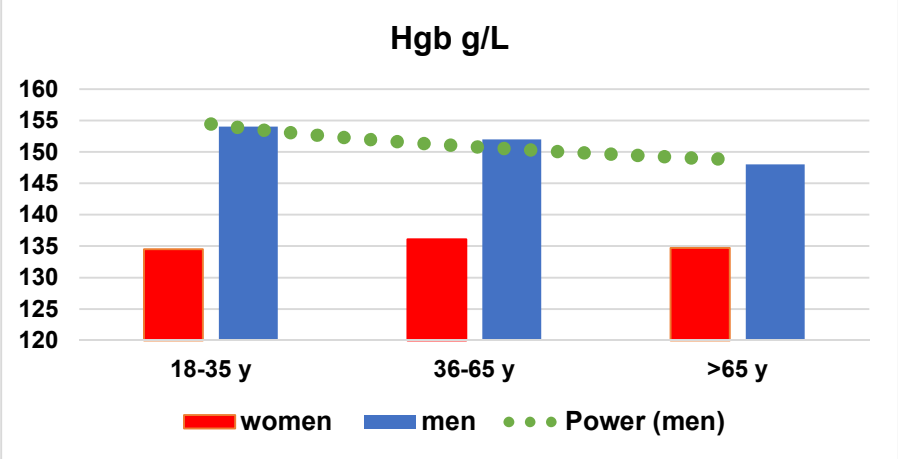


Figure 13. MCV (within the reference limits) according to the sex and age

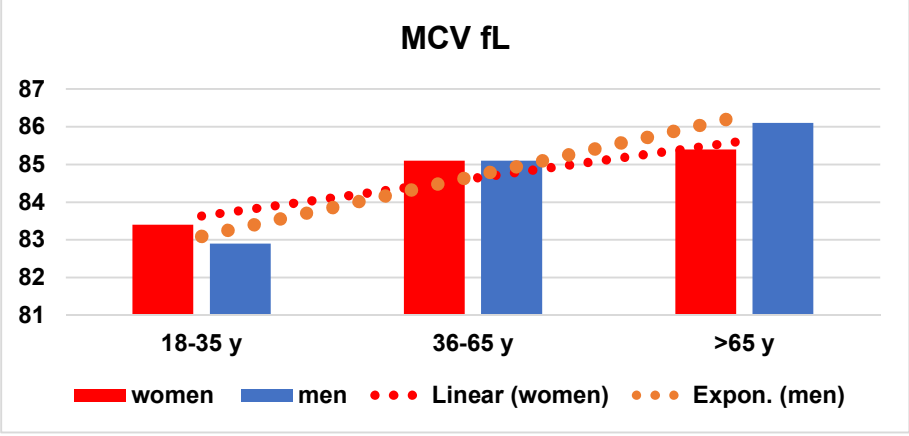


Figure 14. MCH (within the reference limits) according to the sex and age

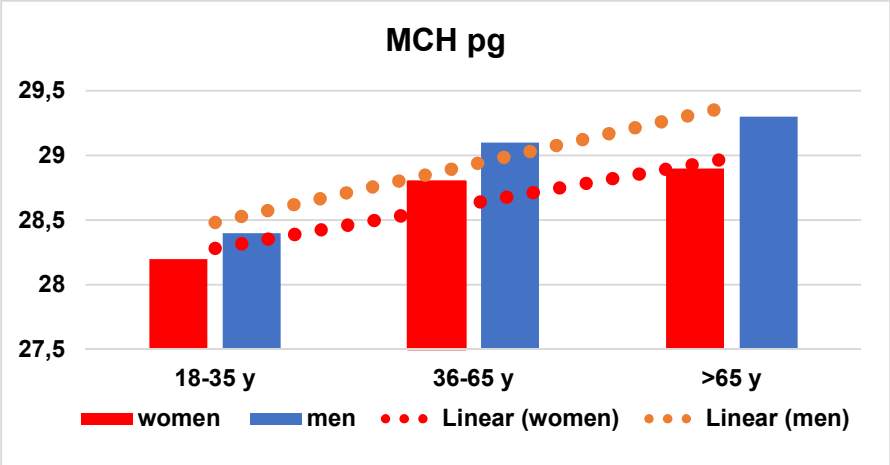
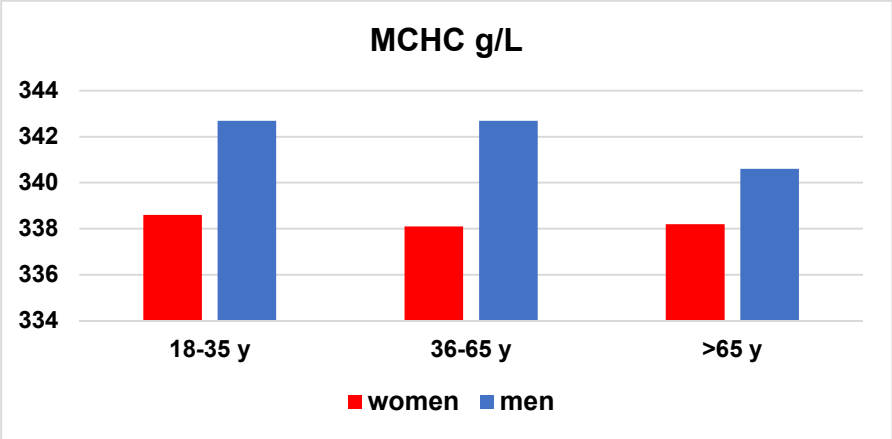


Figure 15. MCHC (within the reference limits) according to the sex and age



The mean value of RBC results within the reference intervals is decreased with age in men (-7.8%), also of Hgb (-4%) and for the same tests there is no change in women. MCV and MCH are increased (+2 - + 4%) for both sexes. MCHC values fall below 1%.

Platelets

Results (mean values ± SD) for Plts are presented for the respective month in the year, for the age and sex (Tables 7 – 9).

Table 7. Number of platelets, within the limits of the reference interval, depending on the sex in different months of the year

	Men		Women	
	n	Platelets		Platelets
Jan	1353	234.2±52.8	1569	253.3±52.1
Feb	1354	234.9±50.2	1515	251.1±51.3
Mar	1552	233.4±50.5	1933	247.8±53.0
Apr	1363	235.8±50.3	1583	250.7±54.3
May	1561	232.5±50.7	1862	253.2±52.3
Jun	1579	232.3±48.2	1007	250.6±51.2
Jul	1342	231.9±51.1	1664	250.1±52.0
Aug	1559	232.0±52.3	1804	249.5±51.8
Sep	1590	233.9±50,6	1959	253.2±52.3
Oct	1789	235.1±50.9	2026	253.2±52.3
Nov	1776	239.8±48.7	2154	253.2±53.0
Dec	1498	239.1±52.4	1751	263.7±50.9

Table 8. Number of platelets, within the limits of the reference interval, depending on the age in different months of the year

ID	18 - 65 years		Over 65 years	
	n	Platelets	n	Platelets
Jan	1499	245.7±54.7	1423	228.3±55.9
Fev	1604	243.7±53.3	1265	230.3±56.1
Mar	2046	244.6±54.7	1439	228.0±54.3
Apr	1723	244.5±56.1	1223	230.755.5±
May	2141	244.4±55.0	1282	231.6±56.5
Jun	1592	244.2±52.1	994	231.9±58.6
Jul	1987	243.1±55.8	1019	230.6±56.8
Aug	2022	241.9±54.0	1341	232.7±56.7
Sep	2199	247.1±55.0	1350	228.4±56.4
Oct	2415	245.7±53.0	1400	231.2±55.9
Nov	2355	248.5±55.1	1575	235.357.3±
Dec	2031	244.9±55.3	1218	233.6±58.1

Table 9. Age and sex-matched platelets mean values of the results within the reference range

Age (years)	men	Platelets x 109/L	women	Platelets x 109/L
18-35	1540	239.9±50.8	1520	258.2±53.4
36-65	9659	238.0±55.1	10895	254.4±55.1
>65	6926	226.1±56.9	8603	239.5±57.9
Total	18125		21018	

Figure 16. Platelets sex-matched annual variations

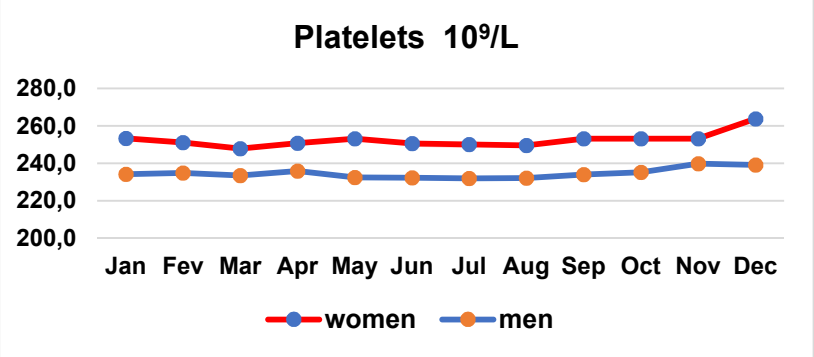


Figure 17. Platelets age-matched annual variations

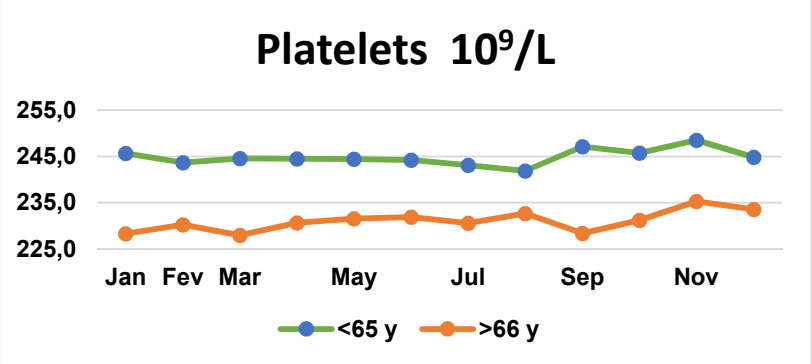
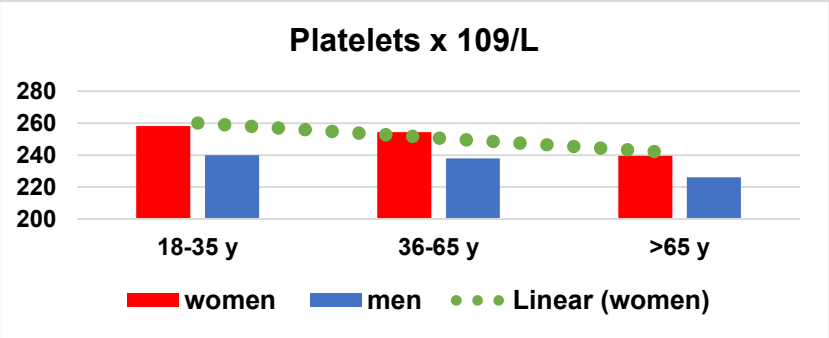


Figure 18. Platelets age- and sex-matched



Women have about 5% higher platelets throughout the year. In younger women, they are higher by + 8% (the age up to 35 years) and the age over 65 y by + 6%. In December, the women's number of thrombocytes are increased by + 4.2% and in men by + 3% compared to the summer.

Discussion

Sources of variation in laboratory results can be pre-analytical, analytical and biological. Pre-analytical and analytical phases are part of the total testing process and the biological variation reflects physiological fluctuations of biomarkers around a homeostatic setting point at a steady state and at a point in time (2). The components of biological variations are within an individual (intra-individual) and between individuals (inter-individual, group). The study of the factors of variation is important to ensure high reliability of the results.

Biological sources of variation, in turn, are often used as factors for stratifying reference intervals. Factors for biological variation are: biological rhythms, homeostasis, age, sex, stage of body development, ethnicity, state of well being, stimuli and socioeconomic impacts (2). The determination of the factors, responsible for biological variations, and the establishment of their relations to reference intervals, and thus to medical decisions are highly complexed. Various European initiatives exist in this direction: EFLM Biological Variation Working Group (BVWG) 2009 and EFLM Biological Variation Task and Finish Group 2015 (EFLM – European Federation of laboratory Medicine).

In the literature, official data on the study of biological variation date back as far as 1835. Science development and the awareness of man as unique individual and simultaneously the awareness about the place of every individual in the whole society, have led to the formulation of the concept of the "average" person as a subject under influence of different

variables with normal distribution (4). Adolphe Quetelet is the first to apply the Gaussian distribution to "measurement" of humans. He suggests that normal distribution of the variations in the populations allows effective selection of artificial or natural information to operate with (5). To date, reference to the biological variation of a number indicators is available on European Federation of Clinical chemistry and Laboratory Medicine platform database (6).

In the present study, the intra-individual seasonal variation using patterns at monthly levels within the reference range are presented. There are surveys for different countries, latitudes, and even continents. Seasonality is established, even at the gene level. Some studies report changes in individual cells. Fluctuations typically span periods of several months.

Several studies have examined the seasonality of hematological indices with different results regarding peaks and troughs (7). These discrepancies have been suggested to be the result of factors such as age and sex. In the large De Jong S. at al. study, the same seasonal patterns are observed for men and women and for different age groups. Seasonal patterns are observed primarily in RBCs and Plts (7).

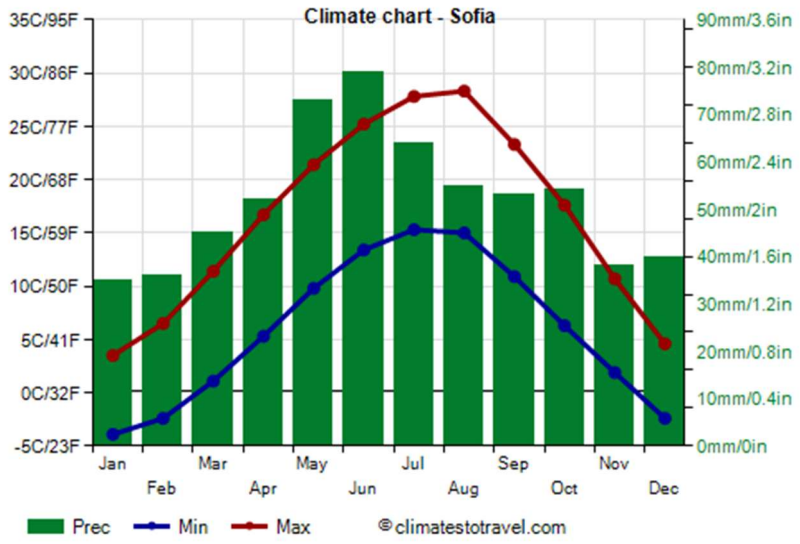
De Jong S. at al. reports that seasonal fluctuations for RBCs and Plts are the same for males and females and for different age groups (8). In 7343 healthy adult blood donors from Cambridge BioResource, United Kingdom, Xaquin Castro Dopico finds low RBCs in December and high in May, in contrast to MCV being high in December and low in May (8). Strikingly, in 4200 healthy individuals from The Gambia (West Africa), these seasonal variations differ distinctly from UK group, peaking during the rainy season (8). In Sofia, in a study of 31,926 patients, RBCs are also with the lowest values in November and December (-5.8% in men and -4.6% in women) compared to the autumn.

The present study demonstrated an impressive growth in the indicators of hemoglobin filling of erythrocytes (MCH, MCHC) during September - December 2022: + 4% for MCHC and + 7% for MCH and for MCV + 2.7%, as well as Hgb in both sexes. It could speculate about the indices for erythrocyte hemoglobin filling to depend on the amount of folate in the food and the limited dietary intake to be responsible for increasing values of the relevant hematology parameters. The lowest values of MCHC and MCH in our group are in September. From October, this rise begins, which suspiciously corresponds with the announcement from the Czech Republic about the highest serum folate values in September (9).

The influence of climate is illustrated by Masayuki Ikeda et al. in a broad measurement of Hgb and RBC during the four seasons of the year in the early 1980s in 15 groups at 7 locations (from Sapporo to Bangkok) in 4 Asian countries with different climates. Both parameters are significantly lower, the outside temperature being higher than 20°C in summer and lower than 5°C in winter. Seasonal variation is absent if the temperature being 25°C - 30°C throughout the year or the work premises are air-conditioned (10). In Bulgaria, there are 4 clearly defined seasons with annual temperature fluctuations between -5 °C in December and January in winter and close to +30 °C in July and August, for Sofia (Figure 19) (11).

Hawkins WW et al. report Hgb values in men and women aged 6 to 98 years old from Halifax, Canada (data since 1954). For men, values are highest in 20s years old individuals. After the age of 50, a progressive decrease in levels is observed, mainly in men (12). In the recent study, Hgb is higher in men by about 11-12% and decreases with age only in men over 65 by 4%, as well as erythrocytes with about 7%. In females, mean Hgb and RBC levels do not significantly change with age.

Figure 19. Climate chart – Sofia



It is reported for Finland population, the Hgb concentration is the lowest in the summer (July) in both men and women (13). In Sofia, the lowest hemoglobin levels are noticed in August. The same results reports an age-dependent change for men in similarity to our data, but Hgb being increased with age for women (13) in difference to our observation for Bulgarian group.

Plts are found significantly higher in winter - autumn period, with a major peak in December - February (+3.4% and +4.6% on average). These results are obtained from the city of Ferrara from the database of Italian Volunteer Association for blood transfusion (AVIS) concerning 10 years (2001-2010) from 170,238 studies (14). The reported number of results, in this present stud within reference limits for a year (2022) is 3926 patients, which is annually comparable to the Italian study. Ferrara is at 44°50'36.46"N, 11°36'31.25"E., and Sofia at 42° 41′ 30″ N and 23° 19′ 39″ E. We observe an increase in platelets in December in women + 4.2% and for men + 3% compared to the summer.

There are several reports by the same Italian team on the autumn-winter preference of acute cardiovascular diseases (14-17) with the risk of pulmonary embolism is estimated to be at 14% (15). Advanced age may reduce seasonal fluctuations in platelets. Thus, in 54 healthy

adults with a mean age of over 80 years old in Belfast, United Kingdom, there is no seasonal variation (16).

In Switzerland, women over 60 have more platelets than men, but a decline is found in men (17).

With advancing age, the number of Plts can change without clear reasons - aging of blood vessels, bone marrow and blood plasma composition (10). We notice at age over 65 yrs the Plts to be lower with about 5% for the whole year. The decline is gradual. In women, the difference in Plts at age up to 35 yrs and over 65 yrs years is respectively -7.8%, and in men -6.1%.

Biino G. et all report similar data - Plts are higher in women, decline with age, rapidly in childhood, stabilize in adulthood, and further decline in old age. Variations in platelet counts are observed in different geographic areas (17).

Seasonal patterns at Cambridge BioResource (UK) are completely different from those observed in an equatorial cohort (Gambia (West Africa), peaking in the rainy season (8).

Conclusion

We can summarize that the values of hematological tests - RBC, Hgb, RBC indexes and Plts correspond to seasonal changes and range from 4 to 8%, less than our reported fluctuations of immune cells in the same cohort (2) . We also observed sex and age differences. Despite these sex and age differences, the seasonal variation does not change.

The potential power of our short observation is suggested by the large number of samples. Our findings may not apply to people under the age of 18, despite our observations across a wide age range, including the elderly. The temporal resolution in this study is limited to 12 months, which may mitigate or miss more detailed seasonal fluctuations in biomarkers - observations should be performed over several consecutive years and should be related to the changing epidemiological setting.

The synchronicity on the one hand and the reciprocity in the RBC changes on the other, is further evidence that these fluctuations are not random but are the result of seasonal rhythmicity.

Biological variation is a fluctuation around a homeostatic point specific to each individual. This variation can be lifetime variation (growth, age, menopause), cyclic variation which may be daily, monthly or seasonal, and random variation. The biological variation is the physiological variation of the analyte in each measurement (18).

From a practical point of view, biological variation has the following applications in medical practice: improvement of analytical reliability of results with improvement of test sensitivity; better recognition of laboratory errors and quality improvement in laboratory work and individualization of medical care (19).

The importance of biological variations is based on their top place in hierarchy for defining the analytical performance specifications, as it outlines at Milan Strategic Conference of EFLM 2014. Biological variation components of measurands specify the effect of analytical quality on general clinical decisions (20). Biological variation data are also substantial for monitoring of the patient status as they are applied in the calculation of the reference change value and individual reference intervals (21). Additional risk - based approach, considering all Milan models (clinical utility, physiological control, state of the art - technologies and EQA/PT schemes), actually up builds all available information also that about biological variations, thus identifying the role of laboratory tests for clinical decisions and outcome (22). All these directions underline the need for deep understanding on the nature of biological fluctuations as a perspective to achieve high quality of clinical laboratory information.

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