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Seasonal variations of white blood cells in single hospital population

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What we call the beginning is often the end. And to make an end is to make a beginning. The end is where we start from.

T. S. Eliot - Little Gidding (1936-1942)

Abstract

Background: Limited number of studies in humans revealed seasonal patterns of variability in white blood cells.

Material and methods: The study population included 151 743 consecutive patients of the Department of clinical laboratory of the Hospital St Ivan Rilsky, Sofia between January 2022 and April 2023. After exclusion of the abnormal tests the re-maining cohort consisted of 31 910 patients - 18 020 women and 13 890 men

Results. We observed a December peak - a 16.6% increase in leukocytes, with the lowest values recorded in April and June. Neutrophils were highest in December compared to May and June - by 19.5%. The annual fluctuation of lymphocytes was slightly different, with higher values a month earlier - from November to March-April, as the difference of June-December was 21%. Monocytes had smallest numbers in June and showed increase with 22% in October and November.

In summary, we found a difference between the lowest and highest values of leukocytes, neutrophils, lymphocytes, monocytes and eosinophils as follows: 16.6 %, 19.5%, 21%, 22% and 28.5% (all within the reference ranges as per protocol). When comparing the values by sex, we found slightly higher (about 10%) monocyte and eosinophil levels in males than in females

Conclusions. There is a clear seasonal fluctuation with an almost perfect match of the annual peaks in values of different types of white blood cells in December while most values are lower in the summer, July being on the bottom. Despite some fluc-tuations with age and sex, both ageing and gender do not seem to affect the seasonal fluctuations in blood cell populations.

Keywords: seasons, neutrophils, lymphocytes, age, sex

Introduction

Over the centuries scientists and scholars studied and documented the rhythms of the life using different techniques and aiming different goals. The circadian and annual rhythms harmonise life, via geographical, planetary, and cosmic conditions. The daylight cycle is the most significant regulator for daily and seasonal rhythms affecting species outside of the equatorial zones (photoperiods). The former is reflected by the periodicity of melatonin secretion from the pineal gland.
Examples for annual rhythms include the pupation of beetles, migration of birds, hibernation, reproduction (mating at specific seasons) and moulting.
Limited number of studies in humans revealed seasonal patterns of variability in white blood cells - most evident in lymphocyte and neutrophil counts. Most interestingly at a population level, human immune parameters varied across season independent of multiple confounding factors. (1)

Material And Methods

The study participants consists of all consecutive patients of the Department of clinical laboratory of the Hospital St Ivan Rilsky, Sofia (both in- and outpatients) between January 1st to 31st of December 2022. To avoid potential confounding bias due to Covid-19 pandemic, we included also the first four months of 2023. During the tested period of 16 months the data of 151 743 consecutive patients was collected. To evaluate the natural annual fluctuations of the haematological parameters, we have implemented a strict exclusion protocol:

1. Age under eighteen
2. Abnormal quantity of leukocytes (leukocytosis or leukopenia)
3. Lymphocytosis and lymphopenia, eosinophylia, monocytosis (regardless if the total number of WBC was within the normal range)

The exclusion criteria for this study resulted in the removal of the most participants. The remaining cohort with different medical conditions and normal WBC with differential count consisted of 31 910 patients - 18 020 women and 13 890 men. There were 20 470 patients under the age of 65, and 11 440 above the age of 65. All studies were performed using an automated haematology analyser AlinityHQ, 5-diff.

Results

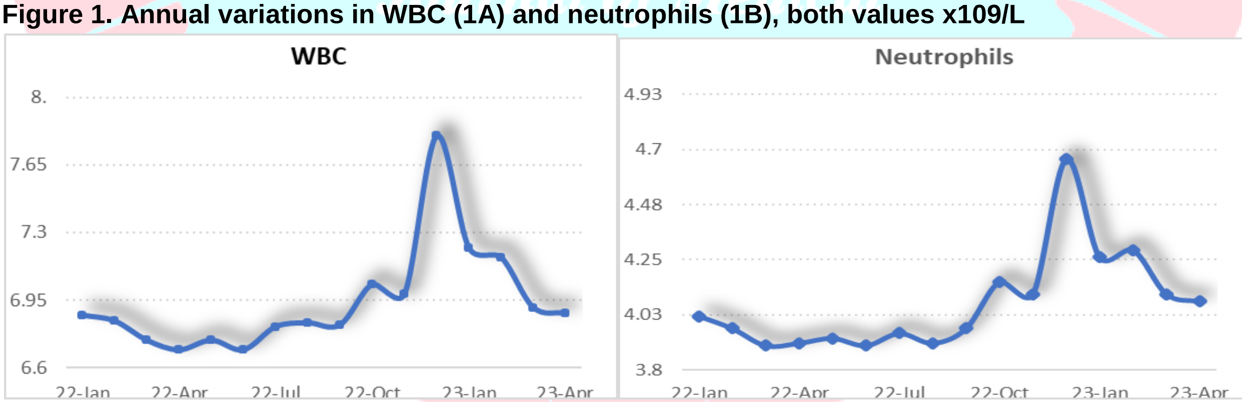
The annual fluctuation of the differential is shown in the **Table 1**

Month	N	Age	WBC	Lympocytes	Monocytes	Neutrophils	Eosinophils
22-Jan	1607	57.57±14.83	6.87±1.67	1.99±0.62	0.51±0.21	4.02±1.20	0.15±0.13
22-Feb	1683	56.57±15.51	6.84±1.66	1.99±0.60	0.50±0.21	3.97±1.20	0.15±0.14
22-Mar	2093	57.90±15.14	6.74±1.64	1.93±0.59	0.52±0.29	3.90±1.19	0.14±0.13
22-Apr	1694	58.12±14.89	6.69±1.65	1.93±0.59	0.51±0.22	3.91±1.19	0.15±0.13

22-May	2122	58.02±14.71	6.74±1.52	1.95±0.54	0.50±0.10	3.93±1.16	0.15±0.10
22-Jun	2228	58.41±14.66	6.69±1.64	1.91±0.56	0.49±0.19	3.90±1.19	0.14±0.12
22-Jul	1834	58.35±15.27	6.81±1.61	1.97±0.60	0.53±0.20	3.95±1.14	0.15±0.13
22-Aug	2045	58.25±14.88	6.83±1.67	1.96±0.60	0.52±0.20	3.91±1.20	0.14±0.13
22-Sep	2235	56.76±14.88	6.82±1.62	2.02±0.60	0.56±0.19	3.97±1.17	0.14±0.12
22-Oct	2319	57.01±15.06	7.03±1.66	2.04±0.60	0.60±0.18	4.16±1.20	0.16±0.15
22-Nov	2362	58.22±14.67	6.98±1.68	2.08±1.19	0.60±0.19	4.11±1.22	0.15±0.15
22-Dec	1470	57.35±15.85	7.80±1.22	2.31±0.76	0.58±0.19	4.66±1.04	0.18±0.16
23-Jan	2517	58.43±16.03	7.22±1.53	2.17±0.67	0.54±0.19	4.26±1.18	0.16±0.16
23-Fev	2315	58.93±14.65	7.17±1.52	2.11±0.59	0.52±0.19	4.29±1.16	0.16±0.13
23-Mar	2131	59.52±14.61	6.91±1.66	2.05±0.60	0.53±0.19	4.11±1.22	0.14±0.11
23-Apr	2368	59.34±14.98	6.88±1.68	2.04±0.61	0.54±0.23	4.08±1.22	0.14±0.12

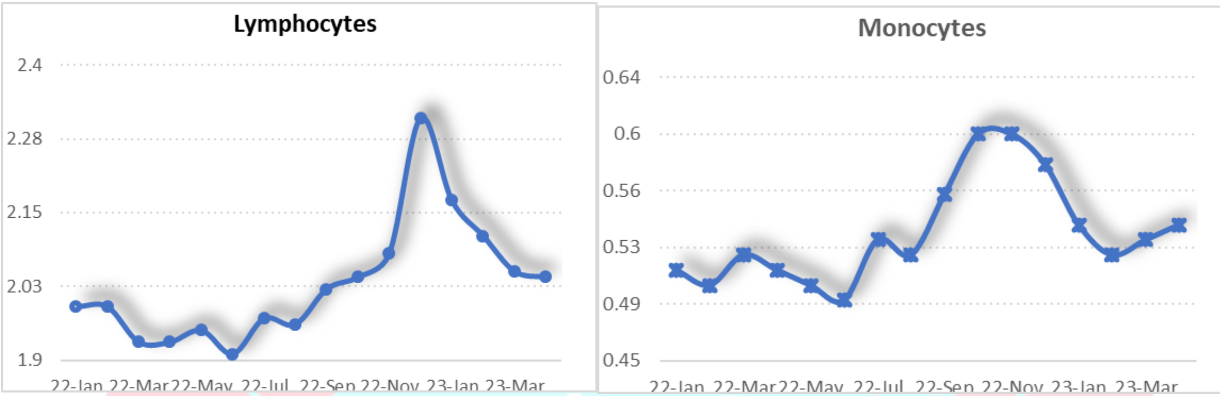
We observe a winter (December) peak - a 16.6% increase in leukocytes, with the lowest values recorded in April and June (figure 1A). Like total white blood cells, neutrophils were highest in December, compared to May and June – by 19.5%. (figure 1B)

Figure 1. Annual variations in WBC (1A) and neutrophils (1B), both values x109/L



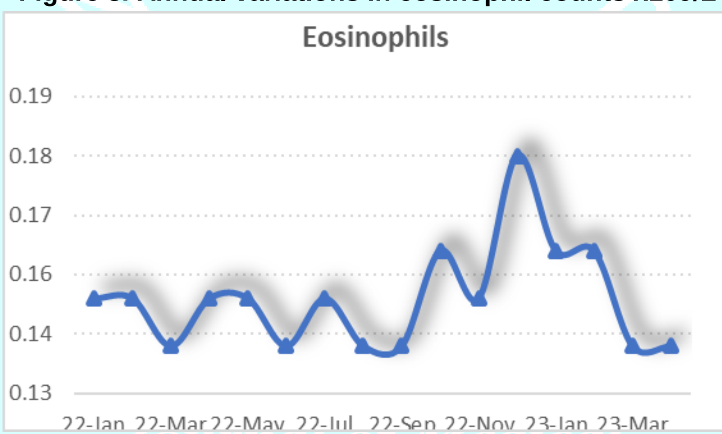
Interestingly, the curve of the annual fluctuation of lymphocytes is slightly different, with higher values a month earlier - from November to March-April, as the amplitude of June-December is 21%. This curve imitates the epidemiology curve of influenza incidence. (Figure 2A). Monocytes had smallest numbers in June and showed increase with 22% in the autumn (October and November) as shown on figure 2B

Figure 2. Annual variations in lymphocytes (A) and monocytes (B) counts x109/L



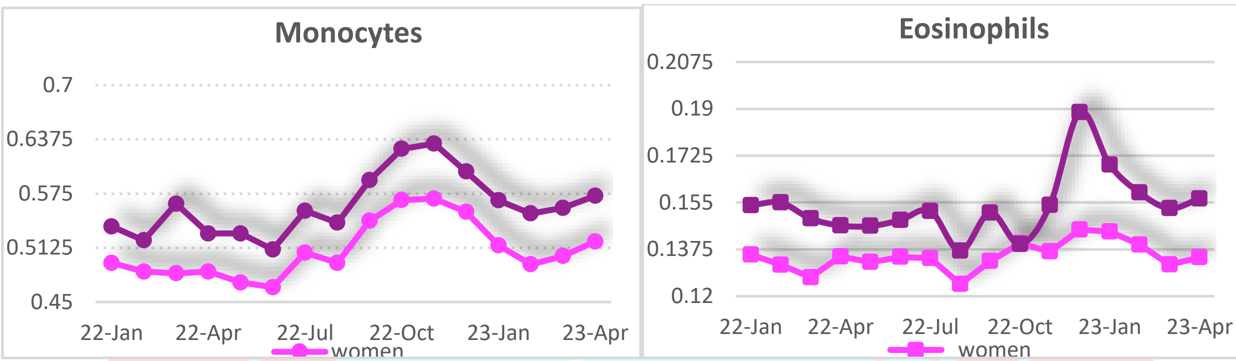
We found also large seasonal fluctuation of the eosinophil counts in the studied cohort. The mean value of the eosinophils were 28.5% higher in December than the lowest levels over the summer months. (Figure 3)

Figure 3. Annual variations in eosinophil counts x109/L



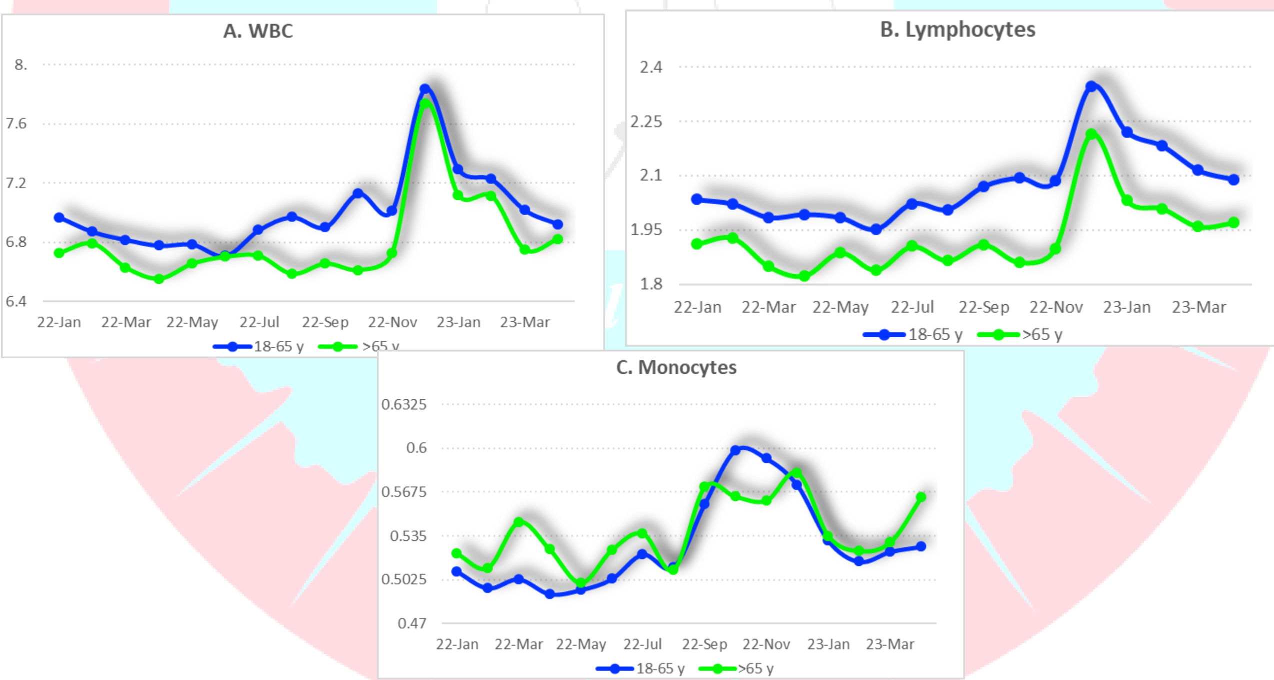
In summary, we found a difference between the lowest and highest values of leuko-cytes, neutrophils, lymphocytes, monocytes and eosinophils as follows: 16.6 %, 19.5%, 21%, 22% and 28.5% (all within the reference ranges as per exclusion criteria of the study).
When comparing the values by sex, we found slightly higher (about 10%) monocyte and eosinophil levels in males than in females. (Figure 4 A and B)

Figure 4. Annual variations in males and females: A. monocytes, B. eosinophils, both x109/L



Accordingly when compared by age, participants over 65 had lower values in almost all cell populations, excluding monocytes which were slightly higher and did not follow the lymphocyte curve, as shown at figure 5.

Figure 5. Annual variations in WBC (A), lympocytes (B) and monocytes (C) in elderly over 65 years old, all levels x109/L



We also found the follows correlation between values of different cell populations and outdoor temperatures and sunlight hours:

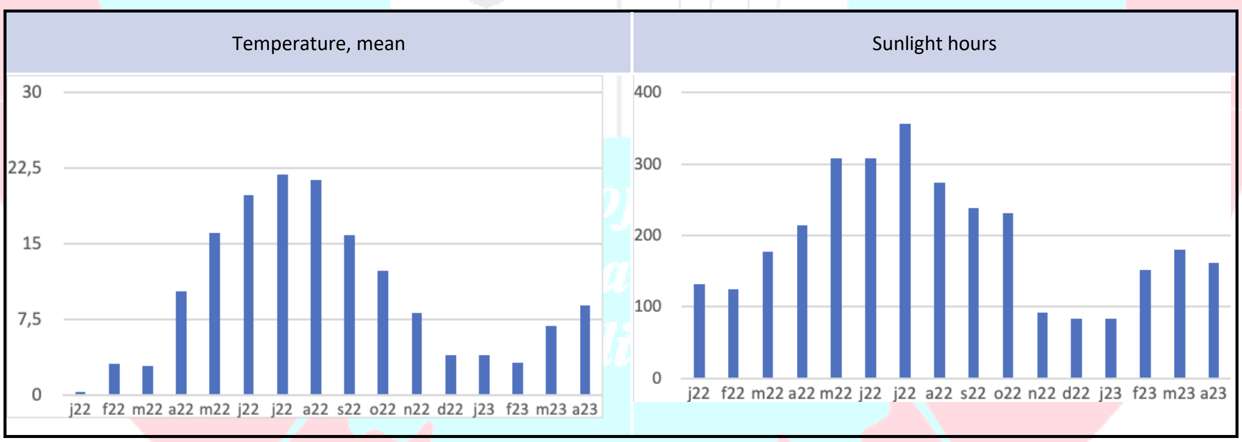
Correlation	WBC	LY	Mo	Neutr	EO	Sunlight hours
Outdoor temperature	–0,428	– 0.463	-0.116	–0,470	– 0,447	0,884
Sunlight hours	-0.595	- 0,666	-0,373	- 0,616	- 0,557	

Discussion

Geography

The axial and orbital rotations of the Earth generate predictable seasonal rhythms of light and darkness. These in turn generate circadian and seasonal oscillations in ambient temperature, food availability, predation, and risk of infections. Most probably, temporal modulation of immune function is one of the innate mechanisms which reflects the environmental challenges, or against infectious diseases such as influenza, whose epidemics generally occur in the winter season, and assured the survival.

The seasonal clock entrains to day length patterns in Northern latitudes. Bulgaria is located at N latitude 42.733883° and W longitude 25.48583° and Sofia is located at N latitude 42.6829987 and W longitude 23.3169994°. Below we present the climatic factors of our latitude, which allows us to compare the obtained results with the commented British studies. The former commented that neutrophil and lymphocyte counts were found to be significantly associated with day length, independent of demographic or lifestyle variables (1).



There are several teams working on annual fluctuations. For example seasonality of blood biomarkers, especially among adults, has been reported before with inconsistent results. We have the availability of data to compare seasonal cell variations in different north latitudes. As far as most of previously reported studies are among healthy populations, we present in- and outpatients with clinical conditions, presented with blood counts within the reference ranges. The seasonal changes in our study showed markable variation around 20 % over seasons, with highest values in December or close months.

Comparing data coming from different parts of the globe, it is most evident that there are striking geographical differences. Even very comprehensive molecular studies presented differences in seasonal gene expressions in Europe, Australia, Iceland etc. Dopico et al. suggested that the daily variables of mean ambient temperature and mean sunlight hours both served as linear predictors of seasonality changes of studied blood markers, suggestive of human environmental adaptation (2). Notably, the Icelandic cohort did not share the same gene expression pattern probably due to differences in the annual photoperiod. Same study revealed winter variations of the PMBC counts, as well as platelets and haemoglobin levels in healthy donors of a BioBank.

Large survey conducted by US National Center for Health Statistics among more that 70 thousands adults showed significant seasonal differences of all CBC variables. Liu et al. reported bortherline significance for eosinophils. The data however showed higher levels of Neutrophils, WBC, and CRP observed in winter-spring than in summer-fall. (3) The authors commented the more pro-inflammatory immune system activation seen in winter-spring could reflect influenza or other viruses. However, the same markers could be involved in the aetiology of many other diseases and discrepancies could not be explained only by the environment (e.g. UV radiation and temperature). Our cohort’s leucocytes also were higher in December to February 2023, slightly higher than in the same period in 2022. The former might be related to the low influenza incidence during the Covid19 pandemic, which strongly affected Bulgaria. (4)

WBC and neutrophils

It is assumed that in the elderly, total WBC will decrease slightly. Blood counts in adult and individuals over eight decades of life were analyzed from a German team and did not found substantial age-related changes in reference intervals (5). Contrary, we observed slightly lower leukocyte values from spring to autumn in persons older than 65 years and almost perfect match the age groups in the winter months. There were no gender difference in leukocytes and neutrophils and lymphocytes. Wyse et al. reported a seasonal pattern of neutrophil counts similarly higher in winter, peaking in Jan with lowest levels recorded in summer with 4% difference in UK cohort (1). The same results were also shown in a US cohort, discussed above (3). Another UK team also confirmed the autumn-winter pattern of neutrophils counts (2). Age is not associated with a change in neutrophil number or activity in the absence of bacterial infection (6). Several studies have shown that neutrophil numbers in the blood and neutrophil precursors in the bone marrow are not lowered in the healthy elderly (7). We also did not observe significant age differences in neutrophils counts.

Lymphocytes

Interestingly, the lymphocytes patterns also peaked in autumn-winter, while being low in the very beginning of the spring in both UK cohorts discussed above These seasonal variations in peripheral lymphocyte counts suggest that humans have some capacity for seasonal regulation of lymphocyte trafficking that could contribute to seasonality in susceptibility to infection (1,2). Alterations in the number and composition of lymphocytes and their subsets in blood are considered a hallmark of immune system ageing or senescence. Brazilian study which compared relatively small numbers of young and elderly adults showed significant reduction in the number of lymphocytes (CD8+, CD2+ and CD3+ cells) in the elderly (8). Similarly, over a five year follow up of 165 subjects aged 24 to 90 years found reduced number of CD4+ and CD8+ T lymphocytes and B lymphocytes in older people, whereas the number of NK, T reg lymphocytes and memory T and B cells increased with ageing (9). Throughout the observation period, our patients over 65 years of age had about 7% lower lymphocyte counts, suggesting greater impairment of the immunity in the analysed elderly population in Bulgaria.

The different studies that track the variability of monocytes show somewhat mixed results. Monocyte counts were higher in winter in some cohorts (10) but not all (3) which showed higher levels in March, but lower in September. Snodgrass et al. found significantly fewer fasting circulating monocytes in older adults, while De Maeyer et al. found an increase with age in circulating CD16+ monocytes (11, 12). No conclusions regarding any relationships between monocytes and age could be drawn from our observations. However, there were 10% higher values of monocytes in males as similar sexual dimorphism was previously also commented by Jisun et al. (13). It is not certain whether there is a relationship with the seasonal variability of monocytes, but these cells have been found to be more inflammatory in nature in males than in females, responding more robustly to metabolic stimuli. We observe higher monocytes in men by about 10%, It has been observed that male monocytes are more inflammatory in nature than female monocytes, since male monocytes respond more robustly to metabolic stimuli (14).

Eosinophils

Despite somehow neglected in the analyses of the peripheral counts, eosinophils are important cells in different inflammatory and metabolic conditions. Most of the big cohort studies from UK did not show seasonal differences. On the contrary, multicenter US study of 713 asthma placebo treated patients showed similar eosinophil levels over the late spring and summer months with maximum January and December values with differences above 27% (15). In our cohort, males had higher eosinophils, which values peaked in December.

This study is the first observation of the seasonal fluctuation of WBC within the reference ranges on our latitude. However we have to discuss several limitations. Our findings does not apply to people under 18 year old due to designed exclusion criteria. The follow up period is relatively short (16 months), which could attenuate or miss more detailed seasonal fluctuations in biomarkers. Our understanding is that similar studies should be further conducted for periods of at least 3 to five consecutive years, also analysing the epidemiological milieu. We also lack data regarding cell subsets, which could be more interesting for further analyses.

Conclusion

In conclusion we could confirm there is a clear seasonal fluctuation with an almost perfect match of the annual peaks in values of different types of white blood cells in December while most values are lower in the summer, July being on the bottom. Winter epidemiological pattern of the viral infections, including influenza, probably affect the increase in white blood cells even within the reference limits. Despite some fluctuations with age and sex, both ageing and gender do not seem to change the seasonal fluctuations.

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