Pediatr Pol 2022; 97 (4): 319-325
DOI: https://doi.org/10.5114/polp.2022.122101
Submitted: 29.06.2022, Accepted: 23.09.2022, Published: 30.12.2022

PEDIATRIA Polska
Polish Journal of PAEDIATRICS

ORIGINAL PAPER

# The associations of complete blood count with Th17 lymphocytes and C-reactive protein levels in overweight and obese children

Dorota Artemniak-Wojtowicz[1], Anna Kucharska[1], Anna Stelmaszczyk-Emmel[2], Małgorzata Rumińska[1], Anna Majcher[1], Beata Pyrżak[1]

[1]Department of Paediatrics and Endocrinology, Medical University of Warsaw, Warsaw, Poland
[2]Department of Laboratory Diagnostics and Clinical Immunology of Developmental Age, Medical University of Warsaw, Warsaw, Poland

## ABSTRACT

**Introduction:** Obesity is considered as a risk factor for chronic low-grade systemic inflammation. This condition could be reflected in changes in complete blood count (CBC) and the presence of inflammatory markers. Assessment of CBC parameters and their correlation with Th17 cells and C-reactive protein (CRP) concentration in overweight/obese children in comparison to normal weight subjects.

**Material and methods:** Twenty-seven overweight/obese and 15 normal-weight children aged 8–18 years were enrolled in the study. The analysis included anthropometric measurements, CBC, biochemical, and immunological parameters. Th17 cells were identified by flow cytometry and defined as CD3+CD4+CD196+IL-17Aic+.

**Results:** In overweight and obese children there were significantly higher counts of leukocyte ($p = 0.04$), lymphocyte ($p = 0.03$), monocyte ($p = 0.02$), erythrocyte ($p < 0.001$), and haemoglobin concentration ($p = 0.005$). In the microscopic smear a lower eosinophil percentage was found in overweight/obese children compared to normal-weight children. Additionally, we detected significant relationships between anthropometric parameters and blood morphology elements, CRP, and Th17 cells in the group of all children, and a statistically significant positive correlation between Th17 frequency and erythrocyte value ($p = 0.01$, $r = 0.38$). Moreover, the band cell value in blood smear correlated with Th17 cells ($p = 0.03$, $r = 0.34$) and CRP concentration ($p = 0.03$, $r = 0.33$) in all children.

**Conclusions:** Obesity affects both white and red blood cell lineages. The finding of significant relationships between pro-inflammatory Th17 lymphocytes, CRP, and immature neutrophils as well as anthropometric markers of obesity may indicate that obesity-induced inflammation is be responsible for these alterations.

KEY WORDS:

**obesity, inflammation, CRP, Th17 lymphocytes, complete blood count.**

## INTRODUCTION

Obesity is considered as a risk factor of such conditions as a cancer, asthma, and some autoimmune diseases [1]. The role of obesity in the development of systemic disorders, including insulin resistance, type 2 diabetes, hypertension, and non-alcoholic fatty liver disease, has been extensively studied in recent years [2]. The role of low-grade chronic systemic inflammation as the basic background of obesity complications is not to be overestimated. Enlarged adipocytes, overexpression of proinflammatory cytokines like interleukin (IL) 6, IL-21, tumour necrosis

ADDRESS FOR CORRESPONDENCE:

Dorota Artemniak-Wojtowicz, Department of Paediatrics and Endocrinology, Medical University of Warsaw, Warsaw, Poland, e-mail: dorota.artemniak-wojtowicz@wum.edu.pl

**TABLE 1.** Age, sex, and anthropometric parameters of overweight and obese (study group) and normal-weight (control group) children participating in the study

| Parameters | Study group ($n = 27$) | Control group ($n = 15$) | $p$-value |
|---|---|---|---|
| Age (years) | 12.67 ±2.55 | 12.65 ±2.64 | ns |
| Gender M/F (%) | 15/12 (56/44) | 8/7 (54/46) | ns |
| BMI [kg/m$^2$] | 29.6 (27.2–33.7) | 18.6 (16.5–20.2) | < 0.001 |
| BMI SDS | 2.3 (1.9–2.5) | 0.1 (–0.5 to 0.3) | < 0.001 |
| WC [cm] | 93.24 ±14.23 | 63.91 ±3.36 | < 0.001 |
| HC [cm] | 105.0 (94.0–109.0) | 80.0 (74.0–87.0) | < 0.001 |
| WHR | 0.88 ±0.055 | 0.80 ±0.05 | < 0.001 |
| WHtR | 0.56 (0.53–0.59) | 0.42 (0.40–0.44) | < 0.001 |

*BMI – body mass index, BMI SDS – body mass index standard deviation score, HC – hip circumference, WC – waist circumference, WHR – waist-to-hip ratio, WHtR – waist-to-height ratio, ns- non-significant*
*Data are presented as median values with interquartile range as appropriate or mean ± standard deviation (SD).*

factor-α (TNF-α) [3], and production of specific chemokines (e.g. monocyte chemoattractant protein-1 [MCP-1]), macrophage infiltration of adipose tissue (AT) [4], and alteration of the immune system (e.g. increase T helper 1 [Th1], decrease Treg cells) [5, 6] contribute to the complex pathogenesis of obesity-induced inflammation. However, the underlying mechanisms of this processes have not been clearly elucidated, and new factors are still being discovered.

Many studies have focused on the role of Th17 lymphocytes in the development and maintenance of obesity-induced inflammation, a few of them also in children [7, 8]. Th17 cells are a subtype of Th lymphocytes that sustain inflammatory milieu in tissues. They are also responsible for the production of antibacterial (extracellular bacteria) and antifungal immunity, and they play an important role in the pathomechanism of autoimmune diseases, chronic inflammation, and allergic processes [9, 10].

It is widely known that inflammation is associated with an increase in the number of leukocytes, both locally and systemically. However, it is not clear which subfractions of peripheral blood cells are typically altered in obesity. Dixon [11] reported that leukocytes are increased in obese patients, Shah [12] described an increase of neutrophils linked to obesity. There is also the observation that the total value of circulating blood cells increases in obese adults with the increase in C-reactive protein (CRP) concentration [13].

Little research has been conducted to assess the relationship between complete blood count (CBC) parameters, and markers of obesity-induced inflammation in children.

The aim of our study was to evaluate the changes in CBC and their correlation with Th17 cells and CRP in overweight and obese children in comparison to normal-weight subjects.

## MATERIAL AND METHODS

We enrolled 27 overweight and obese children (study group) aged 8–18 years: 12 girls and 15 boys, and 15 age- and sex-matched normal-weight children (control group) (Table 1). Anthropometric measurements were taken by one anthropologist. Body weight (kg) and height (cm) were measured using standard methods. Waist and hip circumferences (HC) were measured with a plastic tape measure, according to WHO recommendations [14]. The anthropometric parameters were normalized for calendar age according to a nationally representative group of children aged 3–18 years – the OLAF project [15]. The waist-to-hip ratio (WHR) and the waist-to-height ratio (WHtR) were calculated. The body mass index (BMI) was calculated by dividing the weight (kg) by the square of the height in metres (m$^2$). Obesity was defined by BMI > +2 standard deviation scores (SDS), and overweight was defined as BMI between +1 to +1.9 SDS.

Exclusion criteria for entry into the study were secondary obesity due to hormonal or central nervous system diseases and genetic disorders. All children had no allergy, haematological or chronic disease, or symptoms of acute infection.

All parents and patients older than 16 years gave their informed consent before participating in the study, which was approved by the Bioethics Committee at the Medical University of Warsaw.

### LABORATORY TESTS

In the morning after an overnight fasting period blood samples were obtained by peripheral venipuncture.

Complete blood count was measured in blood collected in EDTA samples using a Sysmex XN 1000i haematological analyser by standard methods.

Analysis included total leukocyte count, neutrophil, lymphocyte, monocyte, eosinophil, basophil count, erythrocyte count, haemoglobin level, values of mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red blood cell distribution width, and platelet count, mean platelet volume, and platelet distribution width.

Additionally, blood smears were assessed manually using a microscope.

The concentration of CRP (mg/dl) was measured by a fixed-point immune-rate method on a Vitros 5600 analyser (Ortho Clinical Diagnostic, Raritan, New Jersey, USA).

Th17 cell evaluation by flow cytometry was as follows: 50µL of fresh venous blood after staining with monoclonal antibodies (according to the manufacturer's instructions – Becton Dickinson Biosciences): anti-CD3 APC-H7; anti-CD4 PE-Cy7; anti-CD196 APC (CCR6) (Becton Dickinson, Franklin Lakes, NJ, USA), were washed (in a washing buffer [0.9% NaCl]), permeabilized ( IntraPrep Permabilization Reagent 1 and 2 (Immunotech SAS, Beckman Coulter Company, 13276 Marseille Cedex 9, France), and stained intracellularly with anti-IL-17A PE monoclonal antibody.

Cells were stored at room temperature before analysis. For staining procedures appropriate isotype-matched controls were used. Flow cytometry was performed on an FACS Canto II flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) using BD FACS Diva 8.0.1 software. Gates were preset, and the measurements were performed blinded for sample identity. Th17 cells were defined as $CD3^+CD4^+CD196^+$ expressing intracellular IL-17A ($CD3^+CD4^+CD196^+IL\text{-}17^{ic+}$). The number of Th17 cells was expressed as a percentage of total $CD3^+CD4^+$ Th cells.

### DATA ANALYSIS

Statistical calculations were performed using SPPS 13.3 software. The normality of the distribution was checked using the Shapiro-Wilk test. Normally distributed data are presented as means and standard deviation (SD), and for data with non-normal distribution, median with interquartile range. To compare overweight and obese children and the control group, Student's *t*-test (parametric data) or the Mann-Whitney *U* test (non-parametric data) was performed, as appropriate. Spearman's rank correlation coefficient was used to perform relationship analyses. A $p$-value < 0.05 was considered statistically significant.

## RESULTS

The characteristics of children included in the study are presented in Table 1.

Complete blood count: the comparison of CBC' parameters in both groups of children are shown in Table 2.

The overweight and obese group had significantly higher leukocyte ($p = 0.04$), lymphocyte ($p = 0.03$), and monocyte counts ($p = 0.02$). The neutrophil value was higher in the study group, but without statistical significance ($p = 0.14$). In the microscopic smear a lower eosinophil percentage was found in overweight/obese children compared to normal-weight children (Figure 1), while the band cell frequency was higher in the overweight/obese group than in the control group, but without statistically significance; median value 1% (0–2) vs. 0% (0–0), respectively ($p = 0.07$). Statistically significantly higher values of erythrocyte ($p < 0.001$) and haemoglobin ($p = 0.005$) were found in the study group. No statistically significant difference was found in platelet value, although it was higher in overweight and obese children ($p = 0.35$).

**TABLE 2.** Comparison of haematological parameters in overweight/obese children (study group) and normal-weight children (control group)

| Parameters | Study group ($n = 27$) | Control group ($n = 15$) | $p$-value |
|---|---|---|---|
| Leukocyte [cells x $10^3$/µl] | 7.14 (5.66–7.93) | 6.0 (5.19–6.86) | 0.03 |
| Neutrophil [cells x $10^3$/µl] | 3.23 (2.64–4.69) | 3.02 (2.12–3.65) | ns |
| Monocyte [cells x $10^3$/µl] | 0.57 (0.45–0.64) | 0.41 (0.38–0.59) | 0.03 |
| Eosinophil [cells x $10^3$/µl] | 0.2 (0.11–0.3) | 0.22 (0.12–0.38) | ns |
| Basophil [cells x $10^3$/µl] | 0.03 (0.02–0.05) | 0.03 (0.02–0.05) | ns |
| Lymphocyte [cells x $10^3$/µl] | 2.63 (2.1–2.87) | 2.00 (1.84–2.60) | 0.02 |
| Erythrocyte [cells x $10^6$/µl] | 5.13 ±0.37 | 4.70 ±0.25 | < 0.001 |
| HGB [g/dl] | 14.0 (13.1–14.7) | 13.00 (12.2–13.5) | 0.005 |
| MCV [fl] | 79.57 ±4.66 | 80.57 ±5.74 | ns |
| MCH [pg] | 26.84 ±2.08 | 27.19 ±2.26 | ns |
| MCHC [g/dl] | 33.85 ±0.97 | 33.74 ±1.12 | ns |
| RDW (%) | 13.20 (12.4–13.7) | 12.6 (12.4–14.1) | ns |
| Platelet [cells x $10^3$/µl] | 283 (223–361) | 260 (245–284) | ns |
| MPV [fl] | 10.62 ±1.26 | 10.27 ±1.56 | ns |
| PDW | 13.42 ±2.2 | 13.06 ±2.43 | ns |

*HGB – haemoglobin, MCH – mean corpuscular haemoglobin, MCHC – mean corpuscular haemoglobin concentration, MCV – mean corpuscular volume, MPV – mean platelet volume, ns – non-significant, PDW – platelet distribution width, RDW – red blood cell distribution width*

*Data are presented as median values with interquartile range as appropriate or mean ± standard deviation (SD).*

*A p < 0.05 was considered significant.*

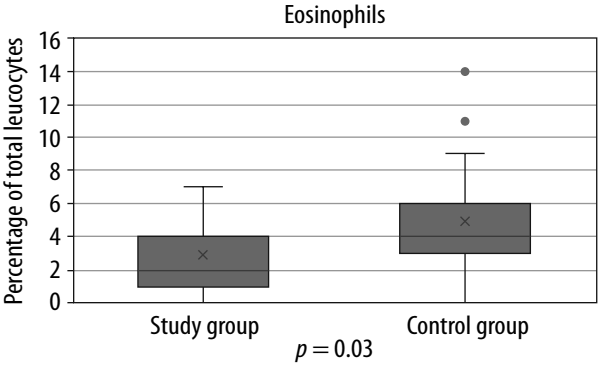


**FIGURE 1.** Statistical analysis of the number of eosinophils (%) in blood smears in overweight/obese children (study group) and normal-weight children (control group). All box plots represent the minimum, the first quartile, the median, the mean, the third quartile, and the maximum. Dots represent outliers

Th17 cells: a statistically significant higher frequency of Th17 cells was found in the peripheral blood of overweight and obese children than in that of normal-weight children; median value 0.097% (0.044–0.289) vs. 0.041% (0.023–0.099), respectively ($p = 0.048$).

C-reactive protein*:* no difference in CRP concentration was found between overweight/obese and normal-weight children.

Correlations*:* we detected statistically significant correlations between anthropometric parameters and blood morphology elements, CRP, and Th17 cells in the group of all children, which is shown in Table 3. Additionally, in overweight and obese children there was a statistically significant positive correlation between the Th17 cell frequency and WHR ($p = 0.005$, $r = 0.54$).

We found a statistically significant positive correlation between Th17 lymphocyte frequency and erythrocyte value ($p = 0.01$, $r = 0.38$) in the group of all children. No correlations were detected between the percentage of Th17 cells and the value of leukocytes or leukocyte subtypes other than basophils ($p = 0.02$, $r = 0.35$). Moreover, a statistically significant correlation was found between the frequency of Th17 lymphocytes and the band cell count in blood smears ($p = 0.03$, $r = 0.34$) in all children. Additionally, the band cell value correlated with the CRP concentration in the overweight/obese group ($p = 0.006$, $r = 0.51$), as well as in the group of all children ($p = 0.03$, $r = 0.33$). Moreover, the CRP concentration correlated positively with the erythrocyte value ($p = 0.02$, $r = 0.44$) in the overweight/obese group. In the group of all children the CRP value correlated with monocyte count ($p = 0.047$, $r = 0.31$).

## DISCUSSION

In our analysis, the number of peripheral leukocytes and the frequency of Th17 cells was significantly higher in overweight and obese children, without any chronic or allergic conditions, which seems to support the theory of obesity-induced inflammation. Other authors presented similar results [16]. Zaldivar *et al.* [17] reported an increase in leukocyte number in overweight children aged 6–18 years without any chronic diseases. Similar results were reported Mărginean [18] in a study involving 164 children. A cross-sectional study of 1024 Iranian adults also supported that the leukocyte count was significantly higher in the obese group in comparison to the of normal-weight group [19]. However, most authors reported that an elevated leukocyte count is mainly related to an increase in monocytes [20], which are more characteristic for chronic inflammation. Zaldivar *et al.* [17] reported significantly higher monocyte count but also neutrophil and T-lymphocyte counts. Other studies evaluating leukocyte subfractions have also shown increases in lymphocytes, neutrophils, and eosinophils in obese subjects [20, 21].

This higher leukocyte count was explained by the influence of increased IL-6 level – pro-inflammatory cytokine production by adipocytes involved in the induction of bone marrow granulopoiesis and the proliferation and differentiation of leukocytes into granulocytes and macrophages [22]. IL-6 also accelerates neutrophil

**TABLE 3.** Correlations between selected blood count parameters, C-reactive protein value, and Th17 cell frequency with adiposity markers in all children

| Parameters | LEUK [x $10^3$/µl] | LYMPH [x $10^3$/µl] | MONO [x $10^3$/µl] | Bands (% of LEUK) | EOS (% of LEUK) | ERY [x $10^6$/µl] | HGB [g/dl] | CRP [mg/dl] | Th17 cells (% of $CD3^+CD4^+$) |
|---|---|---|---|---|---|---|---|---|---|
| BMI [kg/$m^2$] | 0.31* | ns | ns | 0.43** | –0.42** | 0.65** | 0.43** | ns | 0.31* |
| BMI SDS | ns | ns | ns | 0.45** | –0.38** | 0.66** | 0.42** | 0.32* | 0.33* |
| WC [cm] | 0.37* | ns | 0.36* | 0.39** | ns | 0.63** | 0.43** | ns | 0.35* |
| WC SDS | 0.35* | 0.35* | 0.37* | 0.39** | ns | 0.67** | 0.36* | 0.33* | 0.38* |
| HC [cm] | ns | ns | ns | 0.37* | ns | 0.45** | ns | ns | 0.34* |
| WHR | ns | 0.43** | ns | ns | ns | 0.67** | 0.49** | ns | 0.53** |
| WHtR | 0.35* | 0.35* | ns | 0.5** | ns | 0.69** | 0.42** | 0.39* | 0.38* |

*BMI – body mass index, BMI SDS – body mass index standard deviation score, CRP – C-reactive protein, EOS – eosinophil, ERY – erythrocyte, HGB – haemoglobin, LEUK – leukocyte, LYMPH – lymphocyte, MONO – monocyte, WC – waist circumference, WHR – waist-to-hip ratio, WHtR – waist-to-height ratio*
*Data are presented as rs – Spearman correlation coefficient or ns – non-significant.*
**p < 0.05; **p < 0.01*

release from the bone marrow and induces their demargination from the marginal pool. Yoshimura *et al.* [20] in their study found that the peripheral monocyte count significantly correlated with visceral and subcutaneous fat thickness measured by ultrasonography and with body fat mass and percentage body fat calculated by bioimpedance. Additionally, some studies support a positive correlation between changes in leukocyte profile and indices of excessive adiposity, such as BMI, BMI SDS, waist circumference (WC), HC, WHR, or WHtR [17]. In a cross-sectional study of Italian overweight and obese children, an increase in white blood cell (WBC) count quartile was found along with increased mean values of BMI, BMI *z*-score, WC, and WHtR [23]. In the study of Rumińska *et al.* [24] in 99 overweight/obese children aged from 10 to 17.5 years, the WBC count correlated with BMI SDS, the monocytes with WC, and the neutrophils with all anthropometric measurements relating to fatness. In our study, the leukocyte count also correlated positively with the anthropometric parameters including BMI, WC, WC SDS, and WHtR in the group of all children. Furthermore, our data showed a higher value of monocytes in the study group, and a positive statistically significant correlation between the number of monocytes and WC, WC SDS – a widely approved marker of abdominal obesity, which confirmed the relationship between monocytes and abdominal obesity, similarly to previous studies [24]. In our study monocytes also significantly correlated with the CRP concentration in the group of all children. A positive correlation between monocytes and CRP concentration may indicate the involvement of these cells in the inflammatory process occurring in obesity.

Eosinophils, which are known for their role in protecting against parasitic infections, in AT are often distributed close to type II macrophages and secrete type II cytokines such as IL-4, IL-10, IL-13, and TGF-β, which participate in anti-inflammatory immune responses and promote M2 polarization and Th2 differentiation. Wu *et al.* [25] showed a tendency of decreasing eosinophil number in adiposity in obese mice models. Furthermore, they showed that the significant increase in total body fat and percentage fat content in eosinophil-deficient mice on a high-fat diet suggests a protective role of eosinophils against diet-induced obesity [25]. In our study, the eosinophil differential count in the microscopic smear was statistically significantly lower in obese children compared to children with normal weight and correlated with BMI, BMI SDS. This was also found in the studies of Nascimento [21] and Zaldivar *et al.* [17]. The reduction of circulating eosinophil count is acknowledged as a signal of acute infection [26, 27], and eosinopenia is considered as a promising marker for the diagnosis of sepsis [28]. However, lower circulating eosinophil levels in obese children compared to normal-weight ones appear to be associated with a predominance of the proinflammatory over type II response. Further research is required comparing the eosinophil contained in obese and lean AT and peripheral blood to explain this phenomenon.

The next leukocyte subfraction that showed an association with obesity in our study was immature neutrophils (band cells). The presence of band neutrophils > 10% of the total leukocyte count ("bandemia") is often used as an indicator of serious bacterial illness and acute inflammation but is unlikely to be seen in chronic conditions. It is noteworthy that in our research the band cell count was positively correlated with obesity defined using BMI and other anthropometric parameters. Additionally, these cells correlated positively with CRP values. Moreover, we found a positive correlation between band cells and Th17 lymphocytes. The involvement of Th17 cells in the development and maintenance of systemic inflammation in obesity has been demonstrated in both animal and human models [29, 30]. A higher frequency of Th17 cells in overweight/obese children compared to normal-weight ones was confirmed in our study as well as in other papers [7, 8]. The correlation between the frequency of Th17 lymphocytes and anthropometric obesity indicators such as BMI was reported by several authors. In the study of Schindler *et al.* [8] Th17 cell frequencies correlated positively with the absolute BMI. Our study confirmed the positive correlation between Th17 cells and BMI and WHR. The tendency to increase the number of Th17 lymphocytes in obesity could be explained by the pro-inflammatory effect of leptin. Leptin stimulates the proliferative response of T lymphocytes, resulting in an increase in Th1 and suppression of Th2-type cytokine production. Leptin also activates human B lymphocytes to secrete TNF-α, IL-6, and IL-10 through the JAK2, STAT3, p38MAPK, and ERK signalling pathways, and it sustains Th17 pro-inflammatory function [31]. Neutrophils, monocytes, and macrophages are also activated by leptin. Similarly to cytokines such as TNF-α, IL-6, and IL-10, leptin stimulates CRP production in the liver [16, 32].

An increased level of CRP is widely observed in obesity [16, 24]. Some researchers showed a positive correlation between CRP value and anthropometric measurements relating to fatness [24]. Gilbert-Diamond *et al.* [33] in 2614 children aged 5–12 years found that CRP was positively correlated with BMI. However, in NHANES study of 6–11-year-old Mexican American children, the CRP concentration was positively associated with both skin fold sum and skinfold ratio [34]. These results are consistent with our results in which the plasma CRP concentration was correlated with anthropometric markers such as BMI SDS, WC SDS, and WHtR. However, the CRP value did not differ significantly between overweight/obese and normal-weight children. This may be related to the small group of children involved in the study.

Obesity is thought to alter erythropoiesis. Some authors reported that the number of erythrocytes was lower in obese subjects compared to normal-weight ones, often

with a concomitantly lower haemoglobin value [35]. These disturbances are explained by lower iron bioavailability as a result of increased expression of serum hepcidin, which is observed in an inflammatory state [36]. However, there are also contradictory reports that erythrocyte or haemoglobin values were higher in obese subjects compared to normal-weight adults [37]. Similar results were also obtained in a study of children, in wihch the erythrocyte value was significantly higher in the overweight and obese group compared to the control group [18]. In our study the results were in line with the latter data – we found significantly higher values of erythrocyte and haemoglobin in overweight and obese children. These results may be related to increased plasma ferritin level (also an inflammatory marker), which lead to an increase in erythrocyte count [32]. Furthermore, chronic obesity-related hypoxia is considered to lead to AT dysfunction, inflammation, and the development of insulin resistance (IR) [30], and it contributes to the increase of erythropoiesis. The hypothesis that insulin supports *in vitro* and *in vivo* erythropoiesis is not new. Previous studies reported that insulin acts directly on human erythroid progenitors *in vitro* [38] and *in vivo* [39]. Barbieri *et al.* [39] showed in their study in old adults that hyperinsulinemia stimulates erythropoiesis *in vivo*, and insulin and HOMA-IR are positively correlated with the concentration of erythrocyte and HGB. Other authors have obtained similar results [40]. We also found significant correlation in the group of all children between erythrocyte, haemoglobin, and IR markers (data not published). Moreover, we found a positive correlation between erythrocyte value and Th17 cell frequency in the group of all children. In our study, increased erythropoiesis was associated with both IR and inflammation, which appear to be mutually exclusive, because the obesity-induced inflammation, opposite to IR, is thought to support the downregulation of erythropoiesis. Additionally, in some research a correlation was found between erythrocyte, haemoglobin value, and anthropometric parameters including BMI, WC, or WHR, WHtR [40]. Our results also show an association between erythrocyte, haemoglobin values, and WHR, WHtR in overweight/obese children. The occurrence of elevated erythrocyte parameters in obesity may be also partially explained by mild hypercortisolaemia experienced by individuals with excess visceral AT [41]. Nonetheless, in our study the cortisol was not evaluated, so this idea is not supported by our data.

## CONCLUSIONS

Our results confirm the observation that obesity affects haematopoiesis in both white and red blood cell lineages, and these changes are observed in childhood. The finding of a network of significant relationships between pro-inflammatory Th17 lymphocytes, CRP, and immature neutrophils as well as anthropometric markers of obesity may indicate that obesity-induced inflammation is responsible for these alterations.

## DISCLOSURE

The authors declare no conflict of interest.

## REFERENCES


1. Kanneganti TD, Dixit VD. Immunological complications of obesity. Nat Immunol 2012; 13: 707-712.
2. Hamjane N, Benyahya F, Nourouti NG, et al. Cardiovascular diseases and metabolic abnormalities associated with obesity: what is the role of inflammatory responses? A systematic review. Microvasc Res 2020; 131: 104023.
3. Breslin WL, Johnston CA, Strohacker K, et al. Obese Mexican American children have elevated MCP-1, TNF-α, monocyte concentration, and dyslipidemia. Pediatrics 2012; 129: e1180-6.
4. Sun S, Ji Y, Kersten S, et al. Mechanisms of inflammatory responses in obese adipose tissue. Annu Rev Nutr 2012; 32: 261-286.
5. Kucharska AM, Pyrżak B, Demkow U. Regulatory T cells in obesity. Adv Exp Med Biol 2015; 866: 35-40.
6. Wang Q, Wang Y, Xu D. The roles of T cells in obese adipose tissue inflammation. Adipocyte 2021; 10: 435-445.
7. Łuczyński W, Grubczak K, Moniuszko M, et al. Elevated levels of Th17 cells in children with central obesity. Scand J Clin Lab Invest 2015; 75: 595-601.
8. Schindler TI, Wagner JJ, Goedicke-Fritz S, et al. TH17 cell frequency in peripheral blood is elevated in overweight children without chronic inflammatory diseases. Front Immunol 2017; 8: 1543.
9. Miossec P, Korn T, Kuchroo VK. Interleukin-17 and type 17 helper T cells. N Engl J Med 2009; 361: 888-898.
10. Cypowyj S, Picard C, Maródi L, et al. Immunity to infection in IL-17-deficient mice and humans. Eur J Immunol 2012; 42: 2246-2254.
11. Dixon JB, O'Brien PE. Obesity and the white blood cell count: changes with sustained weight loss. Obes Surg 2006; 16: 251-257.
12. Shah TJ, Leik CE, Walsh SW. Neutrophil infiltration and systemic vascular inflammation in obese women. Reprod Sci 2010; 17: 116-124.
13. Chen SB, Lee YC, Ser KH, et al. Serum C-reactive protein and white blood cell count in morbidly obese surgical patients. Obes Surg 2009; 19: 461-466.
14. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. World Health Organ Tech Rep Ser 1995; 854: 1-452.
15. Kułaga Z, Litwin M, Tkaczyk M, et al. Polish 2010 growth references for school-aged children and adolescents. Eur J Pediatr 2011; 170: 599-609.
16. Visser M, Bouter LM, McQuillan GM, et al. Low-grade systemic inflammation in overweight children. Pediatrics 2001; 107: E13.
17. Zaldivar F, McMurray RG, Nemet D, et al. Body fat and circulating leukocytes in children. Int J Obes (Lond) 2006; 30: 906-911.
18. Mărginean CO, Meliț LE, Ghiga DV, et al. Early inflammatory status related to pediatric obesity. Front Pediatr 2019; 7: 241.
19. Jamshidi L, Seif A. Association between obesity, white blood cell and platelet count. Zahedan J Res Med Sci 2017; 19: e4955.
20. Yoshimura A, Ohnishi S, Orito C, et al. Association of peripheral total and differential leukocyte counts with obesity-related complications in young adults. Obes Facts 2015; 8: 1-16.
21. Nascimento H, Rocha S, Rego C, et al. Leukocyte count versus C-reactive protein levels in obese Portuguese patients aged 6–12 years old. Open Biochem J 2010; 4: 72-76.

22. Ulich TR, del Castillo J, Guo KZ. In vivo hematologic effects of recombinant interleukin-6 on hematopoiesis and circulating numbers of RBCs and WBCs. Blood 1989; 73: 108-110.
23. Park JM, Lee DC, Lee YJ. Relationship between high white blood cell count and insulin resistance (HOMA-IR) in Korean children and adolescents: Korean National Health and Nutrition Examination Survey 2008–2010. Nutr Metab Cardiovasc Dis 2017; 27: 456-461.
24. Rumińska M, Witkowska-Sędek E, Artemniak-Wojtowicz D, et al. Changes in leukocyte profile and C-reactive protein concentration in overweight and obese adolescents after reduction of body weight. Cent Eur J Immunol 2019; 44: 307-315.
25. Wu D, Molofsky AB, Liang HE, et al. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. Science 2011; 332: 243-247.
26. Bass DA, Gonwa TA, Szejda, et al. Eosinopenia of acute infection: production of eosinopenia by chemotactic factors of acute inflammation. J Clin Invest 1980; 65: 1265-1271.
27. Gil H, Magy N, Mauny F, et al. Value of eosinopenia in inflammatory disorders: an "old" marker revisited. Rev Med Interne 2003; 24: 431-435.
28. Lin Y, Rong J, Zhang Z. Silent existence of eosinopenia in sepsis: a systematic review and meta-analysis. BMC Infect Dis 2021; 21: 471.
29. Kiran S, Rakib A, Kodidela S, et al. High-fat diet-induced dysregulation of immune cells correlates with macrophage phenotypes and chronic inflammation in adipose tissue. Cells 2022; 11: 1327.
30. Wen J, Liu Q, Liu M, et al. Increasing imbalance of Treg/Th17 indicates more severe glucose metabolism dysfunction in overweight/obese patients. Arch Med Res 2021; 52: 339-347.
31. La Cava A. Leptin in inflammation and autoimmunity. Cytokine 2017; 98: 51-58.
32. Ellulu MS, Patimah I, Khaza'ai H, et al. Obesity and inflammation: the linking mechanism and the complications. Arch Med Sci 2017; 13: 851-863.
33. Gilbert-Diamond D, Baylin A, Mora-Plazas M, et al. Chronic inflammation is associated with overweight in Colombian school children. Nutr Metab Cardiovasc Dis 2012; 22: 244-251.
34. Gillum RF. Association of serum C-reactive protein and indices of body fat distribution and overweight in Mexican American children. J Natl Med Assoc 2003; 95: 545-552.
35. Farhangi MA, Keshavarz SA, Eshraghian M, et al. White blood cell count in women: relation to inflammatory biomarkers, haematological profiles, visceral adiposity, and other cardiovascular risk factors. J Health Popul Nutr 2013; 31: 58-64.
36. Nemeth E, Valore EV, Territo M, et al. Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. Blood 2003; 101: 2461-2463.
37. Cheng HL, Bryant C, Cook R, et al. The relationship between obesity and hypoferraemia in adults: a systematic review. Obes Rev 2012; 13: 150-161.
38. Aoki I, Taniyama M, Toyama K, et al. Stimulatory effect of human insulin on erythroid progenitors (CFU-E and BFU-E) in human CD34+ separated bone marrow cells and the relationship between insulin and erythropoietin. Stem Cells 1994; 12: 329-338.
39. Barbieri M, Ragno E, Benvenuti E, et al. New aspects of the insulin resistance syndrome: impact on haematological parameters. Diabetologia 2001; 44: 1232-1237.
40. Choi KM, Lee J, Kim YH, et al. Koreans-Southwest Seoul (SWS) Study. Relation between insulin resistance and hematological parameters in elderly Koreans-Southwest Seoul (SWS) Study. Diabetes Res Clin Pract 2003; 60: 205-212.
41. Björntorp P, Rosmond R. Obesity and cortisol. Nutrition 2000; 16: 924-936.