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Comparison of leukocyte *IL6* expression in patients with gestational diabetes mellitus (GDM) diagnosed by the Polish Diabetes Association (PDA) 2011 and 2014 criteria

Porównanie ekspresji *IL6* w leukocytach pacjentek z cukrzycą ciążową (GDM) diagnozowanych zgodnie z kryteriami Polskiego Towarzystwa Diabetologicznego z 2011 i 2014 roku

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Abstract

Introduction: Controversial data exist in the literature regarding relationship of IL-6 with gestational diabetes mellitus (GDM), partially resulting from different criteria for GDM classification. In the present study, we revised this linkage by investigating leukocyte *IL6* expression and its associations with clinical characteristics of patients diagnosed by the Polish Diabetes Association (PDA) 2011 and 2014 criteria.
Material and methods: A total of 145 pregnant women underwent 75 g two-hour OGTT, and GDM was diagnosed according to PDA 2011 criteria (GDM/PDA 2011 group; n = 113) and PDA 2014 criteria (GDM/PDA 2014 group; n = 104). *IL6* gene expression was investigated in leukocytes of all participants by using real-time PCR method.
Results: Compared to respective NGT control groups, the GDM/PDA 2011 group exhibited higher FPG, two-hour OGTT, HbA_{1C} and *IL6* expression and lower HDL-C, whereas the GDM/PDA 2014 group had higher FPG, one-hour and two-hour OGTT, HbA_{1C} and HOMA-IR, lower QUICKI-IS, and unchanged *IL6* expression.
No differences in metabolic parameters and *IL6* expression were found between the two GDM groups. Compared to the NGT/PDA 2011 group, the NGT/PDA 2014 group had lower one-hour and higher two-hour OGTT and increased *IL6* expression. With PDA 2014 criteria, *IL6* expression correlated positively with two-hour OGTT in both NGT and GDM groups as well as with LDL-C in NGT group, and negatively with HDL-C in NGT group. With PDA 2011 criteria, no associations were evident in NGT and GDM groups. Nevertheless, significant positive correlation of *IL6* mRNA with two-hour OGTT was observed in the entire study group.
Conclusions: Differences in metabolic phenotypes as well as gene expression and correlation data between GDM and NGT groups, categorised based on PDA 2011 and 2014 criteria, are related to changes in gestational glucose tolerance status resulting from using PDA 2014 criteria. Moreover, our findings support the hypothesis that IL-6 is associated with glucose metabolism during pregnancy. **(Endokrynol Pol 2017; 68 (3): 317–325)**

Key words: *Polish Diabetes Association (PDA); diagnostic criteria; interleukin-6 (IL-6); gestational diabetes mellitus (GDM)*

Streszczenie

Wstęp: W literaturze istnieją kontrowersyjne dane dotyczące związku IL-6 z cukrzycą ciążową (GDM), częściowo wynikające z różnych kryteriów jej klasyfikacji. Celem badania było zrewidowanie istnienia tego związku poprzez badanie ekspresji *IL6* w leukocytach i jej korelacji z charakterystyką kliniczną pacjentek diagnozowanych zgodnie z kryteriami Polskiego Towarzystwa Diabetologicznego (PTD) z 2011 i 2014 roku.
Materiały i metody: 145 ciężarnych poddano 2 godz. OGTT 75 g i GDM diagnozowano zgodnie z zaleceniami PTD z 2011 roku (grupa GDM/PDA 2011; n = 113) i PDA z 2014 roku (grupa GDM/PDA 2014; n = 104). Ekspresję *IL6* badano w leukocytach wszystkich uczestniczek badania z wykorzystaniem qRT-PCR.
Wyniki: W porównaniu z odpowiednimi grupami kontrolnymi NGT, grupa GDM/PDA 2011 wykazywała wyższe wartości glikemii na czczo i w 2 godz. OGTT jak również wyższe poziomy HbA_{1C} i ekspresji *IL6* oraz niższe stężenie HDL-C podczas gdy grupa GDM/PTD 2014 charakteryzowała się wyższymi wartościami glikemii na czczo oraz w 1 i 2 godz. OGTT, wzrostem HbA_{1C} i HOMA-IR jak również obniżeniem QUICKI-IS oraz brakiem zmian w ekspresji *IL 6*. Nie stwierdzono różnic w parametrach metabolicznych i ekspresji *IL 6* między dwoma grupami GDM. W porównaniu z grupą NGT/PDA 2011, grupa NGT/PDA 2014 wykazywała niższe wartości glikemii w 1 h i wyższe w 2 godz. OGTT oraz podwyższoną ekspresję *IL6*. Stosując zalecenia PTD 2014 wykazano, że ekspresja *IL6* korelowała dodatnio z glikemią w 2 godz. OGTT zarówno w grupie NGT, jak i GDM, jak również ze stężeniem LDL-C w grupie NGT oraz ujemnie z HDL-C w grupie NGT. Przy zastosowaniu zaleceń PTD 2011, w grupach NGT i GDM nie obserwowano żadnych korelacji. Stwierdzono, istotną dodatnią korelację między ekspresją *IL6* a glikemią w 2 godz. OGTT w całej badanej populacji.
Wnioski: Różnice w fenotypach metabolicznych jak również w ekspresji genu *IL6* i korelacjach między grupami GDM i NGT, podzielonymi zgodnie z kryteriami PDA 2011 i 2014, są związane ze zmianami stanu tolerancji glukozy w czasie ciąży wynikającymi z zastosowania kryteriów PDA 2014. Uzyskane wyniki potwierdzają także hipotezę, że IL-6 jest związana z metabolizmem glukozy podczas ciąży. **(Endokrynol Pol 2017; 68 (3): 317–325)**

Słowa kluczowe: *Polskie Towarzystwo Diabetologiczne (PTD); kryteria diagnostyczne, interleukina 6 (IL-6); cukrzyca ciążowa (GDM)*

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Introduction

Gestational Diabetes Mellitus (GDM) is an increasingly common complication of pregnancy characterised by carbohydrate intolerance resulting in maternal hyperglycaemia with onset or first recognition during pregnancy. GDM is associated with adverse outcomes for the mother and her offspring [1]. In the long term, GDM women are at elevated risk of developing type 2 diabetes (T2DM), cardiovascular diseases, and metabolic syndrome at a later age [2–4]. Additionally, children born with pregnancy complicated by GDM are at increased risk for the development of obesity and abnormal glucose metabolism during childhood, adolescence, and adulthood [5].

The screening procedures and diagnostic criteria for GDM vary between countries and even within countries, leading to different estimates of the prevalence of GDM, among others. In 1999, the World Health Organisation (WHO) introduced the diagnostic criteria for GDM based on a two-hour 75 g oral glucose tolerance test (OGTT) with fasting plasma glucose (FPG) concentration of ≥ 126 mg/dL (7 mmol/L) or two-hour plasma glucose concentration of ≥ 140 mg/dL (7.8 mmol/L) as the cutoff for diagnosis of GDM [6]. The Polish Diabetes Association (PDA) recommended the use of modified WHO criteria with lowered the FPG cutoff to 100 mg/dL (5.6 mmol/L) [7]. In an attempt to unify the GDM criteria throughout the world, the International Association of the Diabetes in Pregnancy Study Group (IADPSG) proposed in 2010 new diagnostic criteria for GDM based on the results of the Hyperglycaemic Adverse Pregnancy Outcome (HAPO) study, which estimated the risk of maternal and foetal outcomes related to maternal hyperglycaemia [8]. According to the IADPSG guidelines, GDM is diagnosed based on a two-hour 75 g OGTT with the following thresholds: fasting plasma glucose (FPG) ≥ 92 mg/dL (5.1 mmol/L), or one-hour glucose concentration ≥ 180 mg/dL (10.0 mmol/L), or two-hour glucose concentration ≥ 153 mg/dL (8.5 mmol/L) [9]. The cutoff values in the new criteria were set to reflect an odds ratio of at least 1.75 (compared with the population mean) for various adverse foetal outcomes. The IADPSG criteria were endorsed by the WHO in 2013 [10] and PDA in 2014 [11].

Inflammation is a complex process regulated by a cascade of cytokines and growth factors that has been recognised as one of the factors associated with GDM. Among cytokines, interleukin (IL)-6 has drawn much attention not only as an immune-modulating molecule with important functions in the pathology of several inflammation-related diseases such as rheumatoid arthritis (RA), but also as the cytokine linked to type 1 and type 2 diabetes. In this regard, the involvement of IL-6 in the regulation of glucose homeostasis and metabolism by action on skeletal muscle cells, adipocytes, hepatocytes, and pancreatic β-cells has been demonstrated [12]. Interestingly, although IL-6 has been largely seen as a pro-inflammatory cytokine, recent findings suggest its anti-inflammatory role during obesity-associated inflammation and metabolic disorders [12]. Despite the fact that the relationship of IL-6 with GDM has been increasingly investigated in recent years, conflicting findings exist regarding its levels in patients with GDM *vs.* healthy pregnant controls. In this respect, a recently performed meta-analysis of IL-6 levels in patients with and without GDM in the 2nd/3rd trimesters has revealed that among seven analyzed studies, four have shown comparable concentrations of this cytokine in patients with and without GDM, and three have reported its elevated levels in patients with GDM compared with healthy controls [13].

These inconsistencies may be attributed to differences in diagnostic GDM criteria applied (i.e. WHO, National Diabetes Data Group, Canadian Diabetes Association, Australian Diabetes in Pregnancy) and/or gestational age at sampling and/or type of sample (i.e. plasma, serum, culture supernatant).

Since IL-6 is produced by various cells and tissues during diabetic pregnancy, including leukocytes, adipocyte tissue, placenta, skeletal muscle, fibroblast, and endothelial cells, and it is difficult to segregate their significance in IL-6 production during GDM, we have undertaken studies on the contribution of IL-6 production at its transcriptional level in leukocytes obtained from GDM women who were diagnosed by either the PDA 2011 or the PDA 2014 criteria. We also evaluated the impact of the PDA 2011 and 2014 guidelines on correlation analyses done between leukocyte *IL6* expression and the clinical characteristics of patients. In this study we used leukocytes because these cells are well-known to be engaged in modulating inflammatory processes during diabetes and its complications and, on the other hand, they are a good alternative for less accessible metabolic tissues that are difficult to obtain from pregnant women.

Material and methods

Study design

A total of 145 Caucasian pregnant women were enrolled and studied at the Outpatient Diabetological Clinic "OmniMed" in Lodz (Poland) from June 2011 to November 2013. All pregnant women were routinely screened for GDM by a 75-g, two-hour OGTT at 24–28 weeks' gestation or later if it was not possible during this

period, according to the PDA 2011 and 2014 guidelines [7, 11]. Out of all pregnant women recruited, those with family history of diabetes in first-degree relatives, GDM in a previous pregnancy, diabetes diagnosed prior to pregnancy, systemic infection, or taking any drugs known to affect carbohydrate metabolism were excluded from the study.

All clinical investigations were conducted in accordance with the guidelines of the Declaration of Helsinki and were approved by the Bioethics Committee for Research on Humans at the Medical University in Lodz (No. RNN/154/09/KB). All participants provided written, informed consent.

Anthropometric and biochemical measurements

The information on maternal age and pre-pregnancy weight were collected from medical records. Maternal height and weight were measured by standard methods, and body mass index (BMI) was calculated by dividing the weight in kilograms by the height in metres squared.

Plasma glucose was measured by the glucose oxidase method, glycated haemoglobin (HbA_{1C}) was assayed by a latex enhanced turbidimetric immunoassay using specific monoclonal antibodies, and the C reactive protein (CRP) concentration was determined by turbidimetric assay with the use of the cassette COBAS INTEGRA C-Reactive Protein (Latex) according to the manufacturer's instructions (Roche Diagnostics GmbH, Mannheim, Germany). Lipid profiles, including total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), and triglyceride (TG) were determined by enzymatic colorimetric methods (Roche Diagnostics GmbH, Mannheim, Germany). The biochemical assays were carried out with a COBAS INTEGRA analyser (Roche, SA). Plasma insulin level was quantified using Elecsys insulin assay (Roche Diagnostics GmbH, Mannheim, Germany). The homeostasis model assessment (HOMA) index was used to calculate insulin resistance (HOMA-IR) and beta-cell function (HOMA-B) as follows [14]:

HOMA-IR = [fasting insulin (μU/mL) × fasting glucose (mg/dL)]/405 and,

HOMA-B = [360 × fasting insulin (μU/mL)]/[fasting glucose (mg/dL) – 63]

To assess insulin sensitivity, the quantitative insulin sensitivity check index (QUICKI-IS) was calculated as follows: QUICKI = 1/[log(I0) + log(G0)], where I0 is the fasting plasma insulin level (μU/mL) and G0 is the fasting blood glucose level (mg/dL) [15].

Leukocyte isolation and RNA extraction

Leukocytes were isolated from the heparinised venous blood of the subjects (10 mL) as previously described [16, 17]. Total RNA was extracted from leukocytes using Tri Reagent according to the manufacturer's instructions (Ambion, United States). RNA concentration was quantified using a LAMBDA 25 UV spectrophotometer (PerkinElmer, UK), and RNA quality and integrity was assessed by the A_{260}/A_{280} ratio. Samples were kept at -80°C until assayed.

cDNA synthesis and Real-Time Polymerase Chain Reaction

Four μg of high-quality total RNA was converted to cDNA using RevertAid™ H Minus M-MuLV reverse transcriptase kit (Fermentas, Lithuania) according to the manufacturer's recommendations. The cDNA was diluted ten-fold, and 2 μL of cDNA was used to perform RT-PCR using Maxima™ SYBR Green/ROX qPCR Master Mix (2×) (Thermo Scientific, United States) and specific primers for *IL6* and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) as a housekeeping gene. Amplification was carried out on a 7500 Real Time PCR System (Applied Biosystems, United States) with initial denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 60 seconds, 60°C for 60 seconds. All samples were run in duplicate. Amplification of specific transcripts was confirmed by melting curve profiles at the end of each PCR. The specificity of the PCR was further verified by subjecting the amplification products to agarose gel electrophoresis. RNA without reverse transcriptase during cDNA synthesis as well as PCR reaction using water instead of a template showed no amplification.

The threshold cycle (C_t) of each target product was determined, and ΔC_t between target and endogenous *GAPDH* control was calculated as: $\Delta Ct = Ct^{(IL6)} - Ct^{(GAPDH)}$. The relative expression of the *IL6* gene relative to invariant control *GAPDH* was determined using the $2^{-\Delta Ct}$ formula [18].

Statistical analysis

The data are expressed as median values with 25–75% interquartile range. The distribution of analysed biochemical and expression data was checked by the Shapiro-Wilk test. Differences between the groups studied were compared by the nonparametric Mann-Whitney U (Wilcoxon) test. The non-parametric Spearman's rank test was used for analysis of correlation between variables. Additionally, Pearson's parametric correlation was calculated for leukocyte *IL6* expression and two-hour plasma OGTT transformed by the root of the fourth degree to obtain a normal distribution. Statistical analyses were carried out using a commercially available statistical software package (Statistica version *12.5*, StatSoft, Poland), and statistical significance was set at $P < 0.05$.

Table I. *Clinical characteristics of pregnant women diagnosed by the PDA 2011 and 2014 criteria*
Tabela I. *Charakterystyka kliniczna kobiet ciężarnych diagnozowanych zgodnie z kryteriami PTD z 2011 i 2014 roku*

	PDA 2011 guidelines			PDA 2014 guidelines			NGT/ PDA 2011 vs. 2014	GDM/ PDA 2011 vs. 2014
Variables	**NGT/PDA 2011 (n = 32)**	**GDM/PDA 2011 (n = 113)**	**P**	**NGT/PDA 2014 (n = 41)**	**GDM/PDA 2014 (n = 104)**	**P**	**P**	**P**
Age (years)	28.0 (26.0–34.0)	30.5 (27.0–34.0)	0.447	30.0 (26.0–36.0)	30.0 (27.0–33.0)	0.295	0.270	0.519
Pre-pregnancy BMI [kg/m^2]	23.7 (21.4–25.5)	24.4 (21.2–27.9)	0.161	23.4 (20.3–25.5)	24.4 (22.0–27.9)	0.871	0.958	0.923
Pregnancy BMI [kg/m^2]	27.5 (24.5–29.8)	27.6 (25.0–32.7)	0.210	26.6 (23.5–29.8)	28.3 (25.4–32.3)	0.143	0.842	0.782
Body weight gain [kg]	9.5 (6.0–12.0)	8.0 (6.0–11.3)	0.720	7.3 (5.0–10.0)	9.0 (6.0–12.0)	0.285	0.433	0.592
TC [mg/dL]	241.0 (226.0–268.1)	243.0 (224.0–274.0)	0.735	252.0 (227.4–271.1)	241.0 (220.1–275.0)	0.705	0.955	0.991
TGs [mg/dL]	215.0 (177.0–268.0)	212.4 (179.3–271.0)	0.993	206.8 (177.2–249.4)	217.9 (217.9–179.3)	0.775	0.880	0.912
HDL-C [mg/dL]	78.0 (60.0–88.0)	69.8 (55.7–78.0)	0.046*	75.5 (61.5–85.5)	67.9 (55.0–78.0)	0.103	0.779	0.948
LDL-C [mg/dL]	131.0 (112.0–157.0)	138.0 (114.0–153.0)	0.708	135.5 (123.5–155.0)	136.5 (112.0–155.0)	0.789	0.911	0.990
HbA1c (%)	5.3 (4.9–5.6)	5.4 (5.2–5.7)	0.036*	5.3 (5.0–5.5)	5.4 (5.2–5.7)	0.017*	0.770	0.764
FPG [mg/dL]	77.5 (73.0–84.0)	88.0 (79.0–98.0)	< 0.001*	79.0 (74.0–84.0)	89.0 (80.0–99.5)	< 0.001*	0.808	0.594
1 h plasma glucose [mg/dL]	174.0 (160.0–186.0)	180.0 (164.0–202.0)	0.202	161.5 (149.0–172.5)	187.0 (171.0–203.0)	< 0.001*	0.002*	0.110
2 h plasma glucose [mg/dL]	122.0 (106.0–132.0)	156.0 (148.0–176.5)	< 0.001*	139.5 (115.0–147.0)	158.5 (148.5–178.0)	< 0.001*	0.003*	0.814
Insulin [μIU/mL]	6.6 (2.2–9.9)	5.4 (3.0–9.8)	0.903	4.5 (1.6–7.1)	6.2 (3.0–11.5)	0.099	0.336	0.572
HOME-IR	1.2 (0.5–2.0)	1.2 (0.6–2.3)	0.667	0.8 (0.3–1.4)	1.3 (0.7–2.7)	0.034*	0.321	0.520
HOME-β	158.1 (62.2–184.8)	76.8 (54.0–145.9)	0.086	106.4 (40.9–168.4)	90.9 (54.5–184.8)	0.823	0.284	0.621
QUICKI-IS	0.4 (0.3–0.4)	0.4 (0.3–0.4)	0.667	0.4 (0.4–0.5)	0.4 (0.3–0.4)	0.034*	0.321	0.520
CRP [mg/L]	3.7 (2.0–8.7)	3.2 (2.2–5.7)	0.413	3.3 (1.9–6.6)	3.2 (2.2–5.5)	0.747	0.734	0.869

BMI — body mass index; CRP — C reactive protein; FPG; fast plasma glucose; HOMA-B — homeostasis model assessment of B-cell function; HDL-C — high--density lipoprotein; HOMA-IR — homeostasis model assessment of insulin resistance; LDL-C — low-density lipoprotein; QUICKI-IS — quantitative insulin sensitivity check index; TC — total cholesterol; TGs — triglycerides. Data are presented as median and 25–75 interquartile range. *$P < 0.05$ as assessed by the Mann-Whitney U test.

Results

Subject's characteristics

A total of 145 Caucasian pregnant women underwent screening for GDM, and the number of GDM cases identified by the PDA 2011 and 2014 criteria was 113 (i.e. the GDM/PDA 2011 group) and 104 (i.e. the GDM/PDA2014 group), respectively. Out of 113 GDM/PDA 2011 patients, 21 women had the two-hour OGTT values between 140 and 153 mg/dL and therefore their GDM status was changed to NGT when the PDA 2014 guidelines were used. Among 32 subjects classified as the NGT/PDA 2011 group with the PDA 2011 criteria, 11 had plasma glucose values higher than cutoff with the PDA 2014 criteria at one-hour OGTT, and only one case had fasting plasma glucose ≥ 92 mg/dL. Hence, 12 NGT/PDA2011 women were identified as having GDM by the PDA 2014 criteria.

Clinical characteristics of the studied GDM and NGT groups are summarised in Table I. The GDM and NGT groups were comparable for age and parameters of adiposity such as pre- and pregnancy BMI and body weight gain ($P > 0.05$), irrespective of any criteria applied. Compared to respective NGT control groups, the GDM/PDA 2014 women exhibited significantly higher glucose concentrations (i.e. fasting, one-hour and two--hour OGTT), HbA_{1C} and HOMA-IR values, and lower QUICKI indices whereas the GDM/PDA 2011 women

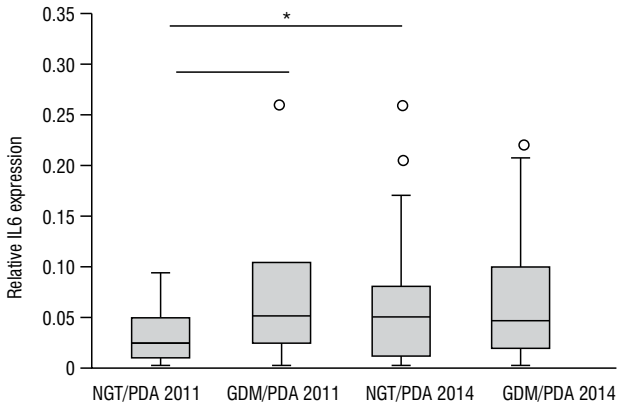

Figure 1. *Comparison of IL6 mRNA expression in the NGT and GDM groups classified based on the PDA 2011 and PDA 2014 criteria*

*Middle line: median; box: interquartile range; whisker: range (excluding outliers). *P < 0.05 as assessed by the Mann-Whitney U test*

Rycina 1. *Porównanie ekspresji IL6 mRNA w grupach NGT i GDM klasyfikowanych na podstawie kryteriów PTD z 2011 i 2014 roku*

*Środkowa linia: mediana; pudełko: zakres międzykwartylowy; wąsy: zakres wartości nieodstających. *P < 0,05 jak oszacowano przy użyciu testu U Mann-Whitneya*

had markedly higher glucose concentrations (i.e. fasting and 2 h OGTT) and HbA_{1C}, and lower plasma HDL-C concentrations ($P < 0.05$). When compared the two NGT groups in respect to clinical parameters, glucose plasma concentrations were significantly lower at 1h and higher at 2h OGTT in the NGT/PDA 2014 group than the NGT/PDA 2011 ($P < 0.05$). Of note, there were no significant differences in clinical parameters between the GDM/PDA 2011 and GDM/PDA 2014 groups ($P > 0.05$).

Leukocyte IL6 gene expression

To examine *IL6* gene expression in peripheral leukocytes from GDM patients diagnosed by the PDA 2011 and 2014 *vs.* respective NGT controls, quantitative real-time PCR studies were performed. As can be seen in Figure 1, a significant increase in leukocyte *IL6* mRNA expression with a 2.24-fold up-regulation was detected in the GDM/PDA 2011 group compared with the NGT/PDA 2011 group ($P<0.05$). Interestingly, there was no difference in the leukocyte *IL6* mRNA level between the GDM/PDA 2014 and NGT/PDA 2014 groups ($P > 0.05$). We compared further leukocyte *IL6* gene expression between the two NGT groups and the two GDM groups. As shown in Figure 1, leukocyte *IL6* mRNA expression was significantly increased (2.08-fold up-regulation) in the NGT/PDA 2014 group compared with the NGT/PDA 2011 group ($P < 0.05$), whereas it did not significantly differ between the two GDM groups ($P > 0.05$).

Correlation studies

To establish whether leukocyte *IL6* expression is associated with clinical characteristics of the patients given in Table I, correlation analyses were made in the NGT and GDM groups, classified according to the PDA 2011 and 2014 criteria, as well as in the entire study group. With the PDA 2014 guidelines, leukocyte *IL6* mRNA positively correlated with two-hour post-load glucose concentration in the NGT/PDA 2014 and GDM/PDA 2014 groups (Spearman's $r = 0.483$, $P = 0.001$ and Spearman's $r = 0.280$, $P = 0.005$, respectively) (Table II). Moreover, *IL6* gene expression associated positively with plasma LDL-C concentration (Spearman's $r = 0.355$, $P = 0.050$) and negatively with plasma HDL-C concentration (Spearman's $r = –0.442$, $P = 0.013$) in the NGT/PDA 2014 group. With the PDA 2011 criteria, no correlation was evident between leukocyte *IL6* mRNA and any of clinical parameters of patients from the NGT/PDA 2011 and GDM/PDA 2011 groups. It is noteworthy that significant positive correlation of leukocyte *IL6* gene expression with two-hour post-load glucose concentration (Spearman's $r = 0.293$, $P < 0.001$ and Pearson's $r = 0.302$, $P < 0.001$) was observed in the entire study group (Table II and Fig. 2).

Discussion

Despite GDM being a common metabolic disease of pregnancy that shares many features of T2DM, including glucose intolerance and insulin resistance, its screening and diagnosis have been controversial over the last three decades, resulting in many inconsistencies in findings obtained from research, clinical, and population studies. To standardise the diagnosis of GDM, the IADPSG recommended in 2010 universal screening of all pregnant women with the 75 g OGTT and proposed new blood glucose thresholds for GDM diagnosis, which were adopted in 2014 by the PDA [9]. Until the end of 2013, the PDA recommended the modified WHO criteria for GDM diagnosis [7].

The primary goal of this study was to compare leukocyte *IL6* gene expression in Polish GDM pregnant women diagnosed using the PDA 2011 criteria (the modified WHO recommendation) and PDA 2014 criteria (the same as those the IADPSG guidelines). A total of 145 pregnant women were included in the present study. By applying the PDA 2011 and 2014 criteria, 113 and 104 pregnant women, respectively, were recognised as having GDM. Out of 113 GDM women, 21 had a two-hour cut-point between 140 and 153 mg/dL, and therefore these cases were classified as NGT by the PDA 2014 criteria. Thus, it is reasonable to assume that since the PDA, like the IADPSG, has increased the two-hour cut-point to 153 mg/dL, many cases of GDM might

Table II. *The Spearman correlation coefficients between leukocyte IL6 gene expression and clinical parameters of pregnant women diagnosed by the PDA 2011 and 2014 criteria*

Tabela II. *Współczynniki korelacji Spearmana między ekspresją genu IL6 w leukocytach, a klinicznymi parametrami kobiet ciężarnych diagnozowanych zgodnie z kryteriami PTD z 2011 i 2014 roku*

	PDA 2011 guidelines				PDA 2014 guidelines					
Variables	**NGT/PDA 2011**		**GDM/PDA 2011**		**NGT/PDA 2014**		**GDM/PDA 2014**		**All patients (NGT + GDM)**	
	r	**P**	**r**	**P**	**r**	**P**	**r**	**P**	**r**	**P**
Age (years)	0.086	0.647	0.106	0.265	0.111	0.489	0.110	0.270	0.108	0.196
Pre-pregnancy BMI [kg/m^2]	–0.124	0.507	0.076	0.430	0.053	0.747	0.073	0.467	0.068	0.420
Pregnancy BMI [kg/m^2]	–0.172	0.354	0.114	0.230	< –0.001	0.998	0.106	0.286	0.090	0.282
Body weight gain [kg]	–0.031	0.870	0.099	0.301	0.059	0.720	0.058	0.565	0.060	0.477
TC [mg/dL]	–0.073	0.724	–0.025	0.841	0.079	0.672	–0.118	0.355	–0.045	0.670
TGs [mg/dL]	–0.302	0.134	–0.041	0.738	–0.084	0.654	–0.082	0.519	–0.090	0.387
HDL-C [mg/dL]	–0.233	0.252	0.031	0.800	–0.442	0.013*	0.027	0.834	–0.107	0.302
LDL-C [mg/dL]	0.084	0.682	0.002	0.988	0.355	0.050*	–0.131	0.324	0.051	0.635
HbA1c (%)	0.316	0.101	–0.022	0.821	0.254	0.118	0.010	0.920	0.079	0.353
FPG [mg/dL]	0.045	0.814	–0.129	0.184	0.084	0.611	–0.034	0.737	0.035	0.687
1-h plasma glucose [mg/dL]	–0.127	0.546	–0.160	0.141	0.093	0.625	–0.133	0.238	–0.117	0.222
2-h plasma glucose [mg/dL]	0.148	0.427	0.125	0.199	0.483	0.001*	0.280	0.005*	0.293	< 0.001*
Insulin [μIU/ml]	–0.285	0.134	–0.043	0.709	–0.183	0.324	–0.057	0.620	–0.073	0.450
HOME-IR	–0.193	0.335	0.000	0.997	–0.098	0.613	–0.016	0.892	–0.004	0.968
HOME-β	–0.257	0.215	0.048	0.694	–0.052	0.797	–0.066	0.588	–0.079	0.446
QUICKI-IS	0.193	0.335	–0.001	0.997	0.098	0.613	0.016	0.892	0.004	0.968
CRP [mg/L]	0.204	0.287	–0.047	0.681	0.052	0.781	–0.057	0.620	–0.022	0.821

r- and P-values are given. Abbreviations as in Table I. * $P < 0.05$ GDM *vs.* NGT

be missed. Interestingly, we also observed that out of 32 women classified as NGT with the PDA 2011 guidelines, 12 were picked up by the PDA 2014 criteria as GDM.

In the present study, we found that shifting from the PDA 2011 criteria to the PDA 2014 guidelines was accompanied by changes in metabolic phenotypes of GDM patients compared to their respective NGT controls. According to this, the GDM/PDA 2011 patients had a higher fasting and two-hour post-load glucose and HbA_{1C} levels and lower HDL-C concentration whereas the GDM/PDA 2014 patients were characterised by a higher fasting, one-hour and two-hour post-load glucose, HbA_{1c}, and insulin resistance assessed by the HOMA-IR method and lower insulin sensitivity calculated from the QUICKI equation. The high HOMA-IR values and low QUICKI-IS values observed in the GDM group are consistent with the findings obtained by Endo et al. [19]. Interestingly, no difference was found in plasma CRP concentrations between the two GDM groups and their respective NGT control groups. CRP is a non-specific acute-phase reactant primarily produced by the liver in response to inflammatory stimuli and its

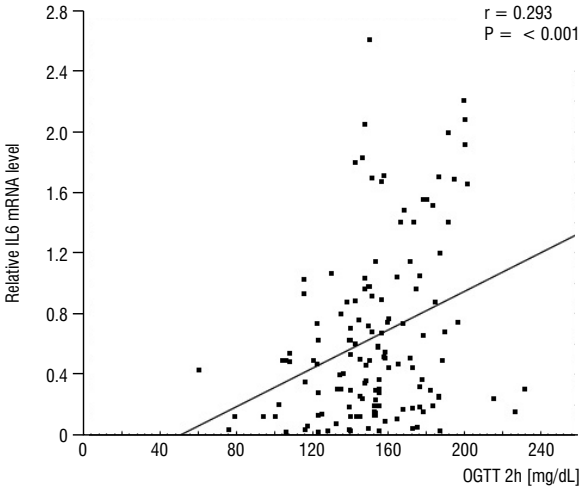

Figure 2. *The positive correlation of leukocyte IL6 mRNA expression with two-hour OGTT glucose plasma concentration in the entire study group of pregnant women (NGT + GDM; n = 145) as assessed by the Spearman's correlation method*

Rycina 2. *Dodatnia korelacja między ekspresją IL6 mRNA w leukocytach a stężeniem glukozy w osoczu w 2 godz. OGTT w grupie wszytkich kobiet ciężarnych uczestniczących w badaniu (NGT + GDM; n = 145) oszacowana przy użyciu korelacji Spearmana*

positive association with GDM was previously reported [20, 21]. On the other hand, the effect of maternal obesity on circulating CRP levels was also demonstrated, suggesting that obesity can be a major determinant of CRP concentration in pregnancy [22]. Therefore, it is more likely that the lack of differentiation in plasma CRP levels between the GDM and NGT groups found in our study, regardless of the diagnostic criteria applied, could reflect no difference in pre-pregnancy BMI between them.

To gain more information about the reasons for the above-described metabolic differences by using the PDA 2011 and 2014 criteria, a comparison of clinical parameters between the two GDM groups and the two NGT groups was performed. Although the results obtained showed a lack of differences in clinical parameters between the GDM/PDA 2011 and GDM/PDA 2014 groups, lower one-hour and higher two-hour glucose values were observed in the NGT/PDA 2014 group *vs.* the NGT/PDA 2011 group. These findings are not entirely surprising given that 21 GDM cases with a two-hour cut-point of 140–153 mg/dL shifted from the GDM/PDA 2011 group to the NGT/PDA 2014 group and 11 NGT cases with one-hour cut-point ≥ 180 mg/dL shifted from the NGT/PDA 2011 group to the GDM/PDA 2014 group.

It is now well accepted that chronic, low-grade, systemic inflammation, shown by alterations in the concentrations of circulating cytokines, is associated with diabetes [23]. Among a panel of diabetes-related cytokines, IL-6 has drawn much attention as a pleiotropic cytokine engaged in metabolic events during T2DM [24], obesity [25, 26], and impaired glucose tolerance [27], but its relevance to GDM has been unclear in previous studies [13]. Indeed, Kuzmicki et al. [28, 29] observed elevated IL-6 concentrations in GDM patients compared with normal pregnant women, whereas Georgiou et al. [30] did not find any difference. Furthermore, a lack of difference in IL-6 release from placenta, adipose tissue, and skeletal muscle between GDM and NGT women was also observed [31]. By contrast, increased *IL6* gene expression was detected in subcutaneous fat, but not in placenta of GDM women *vs.* control subjects [32]. The conflicting results obtained can in part be attributed to differences in GDM diagnostic criteria. Therefore, we renew interest in the connection of IL-6 with GDM in the present study by investigating its expression in leukocytes of diabetic women diagnosed by either the PDA 2011 or 2014 guidelines. Our results revealed that compared to respective NGT control groups, leukocyte *IL6* gene expression was significantly increased in the GDM/PDA 2011 group, but it remained unchanged in the GDM/PDA 2014 group. When leukocyte *IL6* expression was compared between the two GDM groups and the two NGT groups, there was no change in its level between the GDM/PDA 2011 and GDM/PDA 2014 groups, while it was markedly increased in the NGT/PDA 2014 group *vs.* the NGT/PDA 2011 group. These observations suggest that higher two-hour OGTT glucose values can be related to increased leukocyte *IL6* expression in pregnancy because there were more GDM women with a two-hour cut-point of 140–153 mg/dL, who shifted from the GDM/PDA 2011 group to the NGT/PDA 2014 group than NGT women with one-hour cut-point ≥ 180 mg/dL, who shifted from the NGT/PDA 2011 group to the GDM/PDA 2014 group.

In this study, although statistical analyses failed to identify any correlation of leukocyte *IL6* mRNAs with the clinical parameters of the NGT/PDA 2011 and GDM/PDA 2011 patients, significant positive linear relationships were observed between leukocyte *IL6* expression and two-hour post-load glucose concentration in the NGT/PDA 2014 and GDM/PDA 2014 groups. Moreover, the *IL6* mRNA level positively correlated with plasma LDL-C concentration and negatively with plasma HDL-C concentration in the NGT/PDA 2014 group. Nevertheless, there was a significant positive correlation of leukocyte *IL6* gene expression with two-hour post-load glucose concentration in the entire study group, suggesting that a change of leukocyte *IL6* expression can be related with a role of IL-6 in regulating blood glucose levels during pregnancy. In line with this, increased blood glucose after IL-6 infusion in healthy individuals [33], as well as a direct stimulatory effect of IL-6 on hepatic glucose release from glycogen pools by inhibiting glycogen synthase [34], was shown. On the other hand, we cannot exclude the possibility that high glucose conditions may induce the production of greater amounts of IL-6. In this regard, high glucose concentration was demonstrated *in vitro* to induce IL-6 expression at mRNA and protein levels in monocytes through activation of protein kinase C (PKC)-α/β, p38 mitogen-activated protein kinase (p38 MAPK), and nuclear factor-κB (NF-κB) [35]. In addition to glucose action, sugar-derived substances called advanced glycation end products (AGEs) were reported to stimulate IL-6 production in human monocytes, implying that hyperglycaemia-induced oxidative stress could participate in these events [36]. Taken together, considerable future research will be necessary to establish the cause-effect relationship between leukocyte IL-6 expression and glucose metabolism in the context of pregnancy.

Evidence supports IL-6 as an important inducer of CRP expression in hepatocytes by activating the transcription factors STAT3 and CCAAT/enhancer binding protein β (C/EBP β) [37–39]. Despite the fact that several studies have documented the existence of relationship of IL-6 with CRP in GDM women [20, 21], no correla-

tion was seen between leukocyte *IL6* gene expression and plasma CRP concentration among GDM patients in the present study. This observation is not surprising because many molecules, such as IL-1β, IL-4, IL-11, TNF-α, and TGF-β, have been shown to be involved in regulating CRP expression [40–44].

In summary, our comprehensive comparison of clinical characteristics of pregnant women and their leukocyte *IL6* expression, along with its relationships with metabolic parameters of patients, by applying the PDA 2011 and 2014 criteria, points to differences in metabolic, genetic, and statistical data between the GDM groups, identified based on the PDA 2011 and 2014 criteria, and their respective NGT control groups. These differences seem to be highly related to changes in gestational glucose tolerance status resulting from using the PDA 2014 criteria. Importantly, our findings are in line with the hypothesis supporting the relationship of IL-6 with glucose metabolism during pregnancy, but further studies are needed in order to better understand the causal pathway that links IL-6 and plasma glucose levels in pregnant women.

References

1. Forsbach-Sánchez G, Tamez-Peréz HE, Vazquez-Lara J. Diabetes and pregnancy. Arch Med Res. 2005; 36(3): 291–299, doi: 10.1016/j.arcmed.2005.03.001, indexed in Pubmed: 15925019.
2. Ożegowska-Wender E, Sporna M, Zawiejska A, et al. Wykładniki zespołu metabolicznego u kobiet po przebytej cukrzycy ciążowej. Pol Arch Med Wewn. 2007; 117: 457–461.
3. Bellamy L, Casas JP, Hingorani AD, et al. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. Lancet. 2009; 373(9677): 1773–1779, doi: 10.1016/S0140-6736(09)60731-5, indexed in Pubmed: 19465232.
4. Shah BR, Retnakaran R, Booth GL. Increased risk of cardiovascular disease in young women following gestational diabetes mellitus. Diabetes Care. 2008; 31(8): 1668–1669, doi: 10.2337/dc08-0706, indexed in Pubmed: 18487472.
5. Metzger BE. Long-term outcomes in mothers diagnosed with gestational diabetes mellitus and their offspring. Clin Obstet Gynecol. 2007; 50(4): 972–979, doi: 10.1097/GRF.0b013e31815a61d6, indexed in Pubmed: 17982340.
6. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998; 15(7): 539–553, doi: 10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S, indexed in Pubmed: 9686693.
7. Zalecenia kliniczne dotyczące postępowania u chorych na cukrzycę 2011. Stanowisko Polskiego Towarzystwa Diabetologicznego. Diabetol Prakt. 2011; 12(Supl. A): A1–A46.
8. Metzger BE, Lowe LP, Dyer AR, et al. HAPO Study Cooperative Research Group. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med. 2008; 358(19): 1991–2002, doi: 10.1056/NEJMoa0707943, indexed in Pubmed: 18463375.
9. Metzger BE, Gabbe SG, Persson B, et al. International Association of Diabetes and Pregnancy Study Groups Consensus Panel. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care. 2010; 33(3): 676–682, doi: 10.2337/dc09-1848, indexed in Pubmed: 20190296.
10. Diagnostic criteria and classification of hyperglycaemia first detected in pregnancy: a World Health Organization Guideline. Diabetes Res Clin Pract. 2014; 103(3): 341–363, indexed in Pubmed: 24847517.
11. Zalecenia kliniczne dotyczące postępowania u chorych na cukrzycę 2014. Stanowisko Polskiego Towarzystwa Diabetologicznego. Diabetol Klin. 2014; 3(Supl. A): A1–A67.
12. Kristiansen OP, Mandrup-Poulsen T. Interleukin-6 and diabetes: the good, the bad, or the indifferent? Diabetes. 2005; 54 Suppl 2: S114–S124, indexed in Pubmed: 16306329.
13. Gomes CP, Torloni MR, Gueuvoghlanian-Silva BY, et al. Cytokine levels in gestational diabetes mellitus: a systematic review of the literature. Am J Reprod Immunol. 2013; 69(6): 545–557, doi: 10.1111/aji.12088, indexed in Pubmed: 23414425.
14. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28(7): 412–419, indexed in Pubmed: 3899825.
15. Katz A, Nambi SS, Mather K, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab. 2000; 85(7): 2402–2410, doi: 10.1210/jcem.85.7.6661, indexed in Pubmed: 10902785.
16. Wójcik M, Mac-Marcjanek K, Woźniak LA, et al. The association of leukocyte phosphatidylinositol 3-kinase delta overexpression with gestational diabetes mellitus (GDM). Endokrynol Pol. 2014; 65(1): 17–24, doi: 10.5603/EP.2014.0003, indexed in Pubmed: 24549598.
17. Wojcik M, Zieleniak A, Mac-Marcjanek K, et al. The elevated gene expression level of the A(2B) adenosine receptor is associated with hyperglycemia in women with gestational diabetes mellitus. Diabetes Metab Res Rev. 2014; 30(1): 42–53, doi: 10.1002/dmrr.2446, indexed in Pubmed: 23956030.
18. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001; 25(4): 402–408, doi: 10.1006/meth.2001.1262, indexed in Pubmed: 11846609.
19. Endo S, Maeda K, Suto M, et al. Differences in insulin sensitivity in pregnant women with overweight and gestational diabetes mellitus. Gynecol Endocrinol. 2006; 22(6): 343–349, doi: 10.1080/09513590600724836, indexed in Pubmed: 16785160.
20. Wolf M, Sandler L, Hsu K, et al. First-trimester C-reactive protein and subsequent gestational diabetes. Diabetes Care. 2003; 26(3): 819–824, indexed in Pubmed: 12610043.
21. Leipold H, Worda C, Gruber CJ, et al. Gestational diabetes mellitus is associated with increased C-reactive protein concentrations in the third but not second trimester. Eur J Clin Invest. 2005; 35(12): 752–757, doi: 10.1111/j.1365-2362.2005.01574.x, indexed in Pubmed: 16313251.
22. Retnakaran R, Hanley AJG, Raif N, et al. C-reactive protein and gestational diabetes: the central role of maternal obesity. J Clin Endocrinol Metab. 2003; 88(8): 3507–3512, doi: 10.1210/jc.2003-030186, indexed in Pubmed: 12915627.
23. Evangelista AF, Collares CVA, Xavier DJ, et al. Integrative analysis of the transcriptome profiles observed in type 1, type 2 and gestational diabetes mellitus reveals the role of inflammation. BMC Med Genomics. 2014; 7: 28, doi: 10.1186/1755-8794-7-28, indexed in Pubmed: 24885568.
24. Kado S, Nagase T, Nagata N. Circulating levels of interleukin-6, its soluble receptor and interleukin-6/interleukin-6 receptor complexes in patients with type 2 diabetes mellitus. Acta Diabetol. 1999; 36(1-2): 67–72, indexed in Pubmed: 10436255.
25. Mohamed-Ali V, Goodrick S, Rawesh A, et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. J Clin Endocrinol Metab. 1997; 82(12): 4196–4200, doi: 10.1210/jcem.82.12.4450, indexed in Pubmed: 9398739.
26. Spoto B, Di Betta E, Mattace-Raso F, et al. Pro- and anti-inflammatory cytokine gene expression in subcutaneous and visceral fat in severe obesity. Nutr Metab Cardiovasc Dis. 2014; 24(10): 1137–1143, doi: 10.1016/j.numecd.2014.04.017, indexed in Pubmed: 24984824.
27. Müller S, Martin S, Koenig W, et al. Impaired glucose tolerance is associated with increased serum concentrations of interleukin 6 and co-regulated acute-phase proteins but not TNF-alpha or its receptors. Diabetologia. 2002; 45(6): 805–812, doi: 10.1007/s00125-002-0829-2, indexed in Pubmed: 12107724.
28. Kuzmicki M, Telejko B, Szamatowicz J, et al. High resistin and interleukin-6 levels are associated with gestational diabetes mellitus. Gynecol Endocrinol. 2009; 25(4): 258–263, doi: 10.1080/09513590802653825, indexed in Pubmed: 19408175.
29. Kuzmicki M, Telejko B, Zonenberg A, et al. Circulating pro- and anti-inflammatory cytokines in Polish women with gestational diabetes. Horm Metab Res. 2008; 40(8): 556–560, doi: 10.1055/s-2008-1073166, indexed in Pubmed: 18446686.
30. Georgiou HM, Lappas M, Georgiou GM, et al. Screening for biomarkers predictive of gestational diabetes mellitus. Acta Diabetol. 2008; 45(3): 157–165, doi: 10.1007/s00592-008-0037-8, indexed in Pubmed: 18496643.
31. Lappas M, Permezel M, Rice GE. Release of proinflammatory cytokines and 8-isoprostane from placenta, adipose tissue, and skeletal muscle from normal pregnant women and women with gestational diabetes mellitus. J Clin Endocrinol Metab. 2004; 89(11): 5627–5633, doi: 10.1210/jc.2003-032097, indexed in Pubmed: 15531521.
32. Kleiblova P, Dostalova I, Bartlova M, et al. Expression of adipokines and estrogen receptors in adipose tissue and placenta of patients with gestational diabetes mellitus. Mol Cell Endocrinol. 2010; 314(1): 150–156, doi: 10.1016/j.mce.2009.08.002, indexed in Pubmed: 19682537.
33. Tsigos C, Papanicolaou DA, Kyrou I, et al. Dose-dependent effects of recombinant human interleukin-6 on glucose regulation. J Clin Endocrinol Metab. 1997; 82(12): 4167–4170, doi: 10.1210/jcem.82.12.4422, indexed in Pubmed: 9398733.

34. Ritchie DG. Interleukin 6 stimulates hepatic glucose release from prelabeled glycogen pools. Am J Physiol. 1990; 258(1 Pt 1): E57–E64, indexed in Pubmed: 2105658.
35. Devaraj S, Venugopal SK, Singh U, et al. Hyperglycemia induces monocytic release of interleukin-6 via induction of protein kinase c-{alpha} and -{beta}. Diabetes. 2005; 54(1): 85–91, indexed in Pubmed: 15616014.
36. Morohoshi M, Fujisawa K, Uchimura I, et al. The effect of glucose and advanced glycosylation end products on IL-6 production by human monocytes. Ann N Y Acad Sci. 1995; 748: 562–570, indexed in Pubmed: 7695204.
37. Castell JV, Gomez-Lechon MJ, David M, Fabra R, Trullenque R, Heinrich PC. Acute-phase response of human hepatocytes: regulation of acute-phase protein synthesis by interleukin-6. Hepatology 1990; 12:1179-1186.
38. Zhang D, Sun M, Samols D, et al. STAT3 participates in transcriptional activation of the C-reactive protein gene by interleukin-6. J Biol Chem. 1996; 271(16): 9503–9509, indexed in Pubmed: 8621622.
39. Agrawal A, Cha-Molstad H, Samols D, et al. Transactivation of C-reactive protein by IL-6 requires synergistic interaction of CCAAT/enhancer binding protein beta (C/EBP beta) and Rel p50. J Immunol. 2001; 166(4): 2378–2384, indexed in Pubmed: 11160296.
40. Ganter U, Arcone R, Toniatti C, et al. Dual control of C-reactive protein gene expression by interleukin-1 and interleukin-6. EMBO J. 1989; 8(12): 3773–3779, indexed in Pubmed: 2555173.
41. Gabay C, Porter B, Guenette D, et al. Interleukin-4 (IL-4) and IL-13 enhance the effect of IL-1beta on production of IL-1 receptor antagonist by human primary hepatocytes and hepatoma HepG2 cells: differential effect on C-reactive protein production. Blood. 1999; 93(4): 1299–1307, indexed in Pubmed: 9949173.
42. Gordon MS, McCaskill-Stevens WJ, Battiato LA, et al. A phase I trial of recombinant human interleukin-11 (neumega rhIL-11 growth factor) in women with breast cancer receiving chemotherapy. Blood. 1996; 87(9): 3615–3624, indexed in Pubmed: 8611685.
43. Yap SH, Moshage HJ, Hazenberg BP, et al. Tumor necrosis factor (TNF) inhibits interleukin (IL)-1 and/or IL-6 stimulated synthesis of C-reactive protein (CRP) and serum amyloid A (SAA) in primary cultures of human hepatocytes. Biochim Biophys Acta. 1991; 1091(3): 405–408, indexed in Pubmed: 2001421.
44. Taylor AW, Ku NO, Mortensen RF. Regulation of cytokine-induced human C-reactive protein production by transforming growth factor-beta. J Immunol. 1990; 145(8): 2507–2513, indexed in Pubmed: 2170518.