Int J Clin Exp Med 2018;11(9):9865-9871
www.ijcem.com /ISSN:1940-5901/IJCEM0076107

Original Article

Correlation between plasma levels of D-dimer and IL-1, IL-6, and TNF-α in patients with rheumatoid arthritis

Jingjing Ma[1], Yanhui Zhang[2], Yufang Gong[1], Yun Zhu[1], Ming Li[1], Jinmei Zhao[1]

Departments of [1]Rheumatism and Immunity, [2]Gastroenterology, Weifang People's Hospital, Weifang, Shandong Province, China

Received March 15, 2018; Accepted April 27, 2018; Epub September 15, 2018; Published September 30, 2018

Abstract: Objective: The aim of this study was to investigate changes in levels of plasma D-dimer (D-D) in rheumatoid arthritis (RA) patients and analyze its relationship with inflammatory factors interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α). Methods: Plasma levels of D-D, in 138 RA patients and 100 healthy controls, were determined and its correlation with age, rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), IL-1, IL-6, and TNF-α were evaluated. RA patients in the observation group were divided into 3 subgroups based on RA disease activity score 28 (DAS28). There were 52 cases in group A (DAS28>5.1), 42 cases in group B (3.2<DAS28≤5.1), and 44 cases in group C (1.6<DAS28≤3.2). Results: Plasma D-D levels in the RA group were significantly higher than those of healthy control groups (P<0.01). In addition, plasma levels of D-D in groups A and B were significantly higher compared to those in group C (both P<0.01). Moreover, in all RA patients, D-D levels had a significantly positive correlation with age, ESR, RF, C-reactive protein, IL-1, IL-6, and TNF-α (all P<0.01). Conclusion: D-D can be used as a nonspecific inflammatory response index for clinical diagnosis of RA and as a guide for clinical treatment of this disease.

Keywords: D-dimer, interleukin-1, interleukin-6, rheumatoid arthritis, tumor necrosis factor-α

Introduction

Rheumatoid arthritis (RA), a common disease of the elderly, is an autoimmune disease mediated by a variety of immune cells and secreting cytokines. RA mainly manifests as synovitis and is considered a typical chronic inflammatory disease [1]. Prevalence of RA has ranked at the top of connective tissue diseases, with an incidence of about 1% of the total population. However, incidence of RA in China is over 30% of total cases worldwide [2, 3]. A typical chronic autoimmune disorder, RA may also cause several other diseases including subcutaneous nodules, pericarditis, and peripheral neuropathy. RA greatly increases the possibility of disabilities and severely affects patient quality of life. Moreover, RA imposes a heavy economic and mental burden on the families of patients, endangering the lives of RA patients [4, 5].

The main manifestation of RA involves proliferation of synovial fibroblasts, resulting in the secretion of a large amount of synovial fluid and formation of pannus [6]. One study demonstrated that T lymphocytes are involved in the pathogenesis of RA but other studies have shown that T-cells promote secretion of inflammatory factors [7]. A member of the interleukin family, interleukin-1 (IL-1) mainly exists in the forms of IL-1α and IL-1β, multifunctional cytokines playing important roles in tissue repair and immune responses [8]. Moreover, interleukin-6 (IL-6) plays a key regulatory role in cell differentiation, proliferation, and apoptosis and is expressed by many types of cells including macrophages, B-cells, and T-cells [9]. Tumor necrosis factor-α (TNF-α) is a polypeptide cytokine secreted by mononuclear macrophages. It serves as an immune protective medium in the body and participates in the process of immune damage in humans [10]. In a previous study, it was shown that activation of the NF-κB pathway in RA patients resulted in the release of several inflammatory factors (e.g., TNF-α and IL-6) [11]. D-dimer (D-D) is produced by the degradation

of cross-linked fibrinogen and its expression has been shown to be related with joint activity in RA patients. Long-term high expression of D-D has been associated with poor RA prognosis [12].

In this study, expression of D-D in RA was evaluated and its correlation with inflammatory factors IL-1, IL-6, and TNF-α was investigated.

Materials and methods

General data

A total of 138 RA patients were enrolled. They were treated in Weifang People's Hospital between March 2015 to March 2016. All patients met RA diagnostic criteria issued by the 2015 edition of American College of Rheumatology and the European League Against Rheumatism. RA patients included 45 males and 92 females, with ages ranging from 33 to 75 years old and mean age of 56.4±13.5. Patients were initially placed in an observation group, then divided into 3 subgroups based on RA Disease Activity Score28 (DAS28; DAS28 = 0.56 * √ (number of joints with pain) + 0.28 * √ (number of swollen joints) + 0.70 * lnESR + 0.014 * health status score): 52 cases in group A (DAS28>5.1), 42 cases in group B (3.2<DAS28≤5.1), and 44 cases in group C (1.6<DAS28≤3.2). In addition, 100 healthy volunteers (35 males and 65 females aged 27-77 years with mean age of 55.6±12.7), visiting Weifang People's Hospital for physical examination during the study period, were included as the control group. This study was approved by the Medical Ethics Committee of Weifang People's Hospital and signed consent was obtained from all subjects.

Inclusion criteria: Patients over 18 years of age that had not taken non-steroidal anti-inflammatory drugs and immunosuppressive drugs, recently; No blood relationship between patients; No familial genetic diseases; No memory impairment, autism, and hearing impairment.

Exclusion criteria: Patients suffering from hypertension, respiratory diseases, and digestive system diseases; No surgery performed in the past 3 years; Patients that did not cooperate with treatment or follow up visits; Patients with incomplete clinical information.

Main reagents and instruments

The main reagents and instruments were the D-D detection kit, rheumatoid factor kit, C-reactive protein (CRP), and enzyme-linked immunosorbent assay (ELISA) kit (all purchased from Shanghai Yu Bo Biological Technology Co., Ltd.).

Detection methods

Blood collection was carried out in the morning, after fasting. A total of 3 mL fasting venous blood was collected in a sodium citrate anticoagulation tube and mixed. Subsequently, expression of D-D (immunoturbidimetry) and rheumatoid factor (RF, rate nephelometry) was determined using Hitachi 7600 automatic biochemical analyzer. Moreover, levels of CRP and erythrocyte sedimentation rate (ESR) were determined using an immunoturbidimetric instrument and an automatic ESR analyzer, respectively.

ELISA method: For each patient, a total of 3 mL of venous blood was collected in an EDTA-k tube and centrifuged at 4°C and 3,000 rpm for 5 minutes. Supernatant was collected into an EP tube and stored at -80°C for future use. To determine expression levels of IL-1, IL-6, and TNF-α in the blood samples, ELISA assays were performed, in strict accordance with manufacturer instructions (Beyotime Biotechnology Research Institute). Briefly, a coating solution was used to achieve the required concentration of the surface antigen of hepatitis B virus, which was subsequently added into the plate with 200 μL in each well. The plate was then incubated overnight at 4°C and washed once with double distilled H_2O. Next, wells were blocked by the addition of 200 μL blocked solution (Beyotime Biotechnology Research Institute) into each well and incubated at room temperature for 1 hour. Subsequently, blood samples were added into appropriate wells. A negative control group, positive control group, and blank control group were established by addition of 50 mL. After adding the enzyme-labeled antibody provided with the kit, the plate was mixed, sealed, and incubated at 37°C in a constant temperature water bath for 45 minutes. Next, the plate was washed 5 times for 30 seconds - 1 minute using a premade cleaning fluid (Beyotime Biotechnology Research Institute). Finally, samples were subjected to color development by addition of 100 μL of substrate into each well and the

Table 1. General data

	Observation group (n = 138)	Control group (n = 100)	t/χ^2	P
Gender			0.14	0.69
Male	45	35		
Female	93	65		
Age (years)			0.20	0.66
>55	65	50		
≤55	73	50		
BMI (kg/m^2)	23.54±1.68	23.60±1.75	0.27	0.79
WBC ($10^9/L$)	4.58±1.73	4.50±1.66	0.36	0.72
Hb (g/L)	148.36±35.54	149.02±34.64	0.14	0.89
History of diabetes mellitus			0.29	0.59
Yes	85	65		
No	53	35		
Degree of education			0.09	0.77
<High school	62	43		
≥High school	76	57		
Smoking			0.46	0.50
Yes	50	32		
No	88	68		
Excessive drinking			1.10	0.29
Yes	10	4		
No	128	96		
Domicile			0.11	0.74
Village	77	58		
City	61	42		

Note: BMI, body mass index; WBC, white blood cell; Hb, hemoglobin.

plate was incubated at 37°C for 15 minutes. The reaction was terminated by addition of 50 μL of 2 mol/L sulfuric acid into each well and color development was visualized within 15 minutes.

Statistical methods

SPSS 22.0 software (Chicago, IL, USA) was used to analyze collected data and GraphPad Prism 5 software (Cabit Information Technology Co., Ltd.) was used to plot graphs. All measurement data are presented as mean ± standard deviation ($\bar{x}$ ± sd) and analyzed using t-tests. All count data are presented using percentage (%) and evaluated using Chi-square tests. When the variance was homogenous, overall comparisons were performed using F tests and multiple comparisons were performed using least significant difference method. In addition, when variance was not homogenous, overall comparisons were performed using the Welch-Aspin test and multiple comparisons were performed using Dunnett's T method with Spearman's linear correlation analysis. *P* values of less than 0.05 were considered statistically significant.

Results

Analysis of basic patient characteristics

Table 1 shows no significant differences in terms of sex, age, and other indicators of routine blood tests between the control group and observation group (all P>0.05).

Comparison of expression levels of D-D, CRP, ESR, and RF between groups

Comparison between the two groups of patients demonstrated that expression levels of D-D, CRP, ESR, and RF were significantly lower in the observation group than normal control group (all P<0.05). See **Table 2**.

Expression levels of D-D in different subgroups of the observation group

Using the DAS28 score, RA patients in this study were divided into subgroups A (n = 52), B (n = 42), and C (n = 44). When comparing D-D expression levels in these groups, it was found that overall expression of D-D was significantly different between groups (F = 66.62, P = 0.01). Moreover, significant differences were observed between groups A and B (t = 2.17, P = 0.03), between groups A and C (t = 4.28, P = 0.01), and between groups B and C (t = 2.48, P = 0.02). In addition, compared to D-D expression levels in the control group, expression levels in groups A, B, and C were higher (t = 16.35, P = 0.01; t = 11.66, P = 0.01; t = 7.67, P = 0.01 respectively), as shown in **Table 3** and **Figure 1**.

Analysis of correlation between D-D expression in the observation group and clinical indexes in RA patients

In the observation group, D-D expression was significantly correlated with clinical indexes in

Table 2. Comparison of expression levels of D-D, CRP, ESR, and RF between the two groups

	Observation group (n = 138)	Control group (n = 100)	t	P
D-D (μg/L)	1,684.86±984.50	394.65±188.41	12.93	0.01
CRP (mg/L)	87.54±43.82	4.15±2.44	18.99	0.01
ESR (mm/H)	73.88±27.21	22.58±6.84	18.43	0.01
RF (IU/mL)	324.32±258.60	21.11±6.84	11.72	0.01
IL-1 (pg/mL)	7.84±0.98	1.10±0.15	68.16	0.01
IL-6 (pg/mL)	45.88±28.94	2.25±0.71	15.06	0.01
TNF-α (pg/mL)	86.55±49.51	22.37±13.37	12.63	0.01

Note: D-D, D-Dimer; CRP, c-reactive protein; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor; IL-1, interleukin-1; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.

Table 3. Expression levels of D-D among different subgroups

	Case	D-D (μg/L)	F	P
Group A	52	1,882.62±874.65	66.62	0.01
Group B	42	1,484.53±892.68		
Group C	44	1,032.47±784.84		
Control group	100	394.65±188.41		

Note: D-D, D-Dimer.

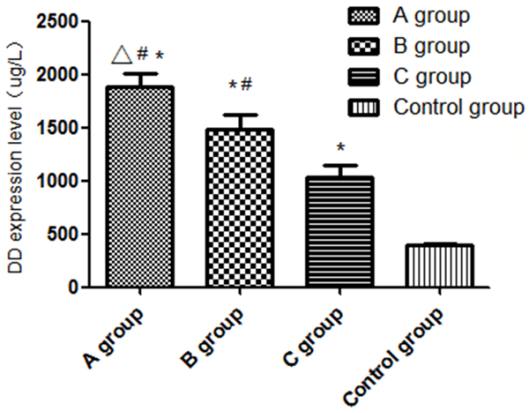

Figure 1. Expression levels of D-D among different subgroups. [Δ]The expression level of D-D in group A was statistically different from that in group B (t = 2.17, P = 0.03); [#]expression level of D-D in group A was statistically different from that in group C (t = 4.28, P = 0.01); [#]expression level of D-D in group B was statistically different from that in group C (t = 2.48, P = 0.02); [*]expression level of D-D in group A was significantly different from that in the control group (t = 16.35, P = 0.01); [*]expression level of D-D in group B was significantly different from that in control group (t = 11.66, P = 0.01); [*]expression level of D-D in group C was significantly different from that in the control group (t = 7.67, P = 0.01).

RA patients, including age, CRP, ESR, RF, IL-1, IL-6, and TNF-α (all P<0.05). As shown in **Table 4**, gender, body mass index, white blood cell count, hemoglobin, history of diabetes mellitus, education background, smoking history, alcohol drinking history, and place of residence were not different between groups (all P>0.05).

Discussion

RA is the most disabling arthritic disease. However, the underlying mechanism of the pathogenesis of RA remains elusive [13]. Currently, it is believed that the pathogenesis of RA involves activation of $CD4^+$ T-cells that subsequently bind to tissue-related cells (major histocompatibility complex class-II, antigen presenting cells), possibly causing activation of synovial macrophages and leading to increased secretion of several inflammatory factors (IL-1, IL-6, and TNF-α) [14].

A marker of cross-linked fibrin, D-D is a product generated by the dissolution of fibrin after blood coagulation with a low molecular weight. Therefore, D-D can be used as an early indicator of coagulation dysfunction [15]. However, in recent years, increasing evidence has shown that D-D not only exists in the process of thrombosis and coagulation but also in the process of traumatic stress and chronic inflammation [16]. In addition, a previous study demonstrated that expression of D-D plays an important role in RA development [17]. In this present study, D-D expression in serum between healthy subjects and RA patients were compared, showing that D-D levels in serum obtained from healthy subjects were significantly lower than in RA patients. These findings indicate that D-D expression might be used as a diagnostic index for RA.

IL-1 is a secretory product of mononuclear macrophages. High levels of IL-1 have been detected in synovial fluid and serum obtained from RA patients. High levels of IL-1 are associated with cartilage injury in RA patients. In addition, IL-1 induces T-cells and B-cells to produce several cytokines and antibodies [18]. A small molecule polypeptide, IL-6 plays a key regulatory

Table 4. Correlation analysis between expression of D-D and clinical indexes

	r	P
ESR	0.43	0.01
RF	0.58	0.01
IL-1	0.78	0.01
IL-6	0.82	0.01
TNF-α	0.82	0.01
Age	0.25	0.01
CRP	0.58	0.01
Gender	0.85	>0.05
BMI	0.51	>0.05
WBC	0.69	>0.05
Hb	0.52	>0.05
History of diabetes mellitus	-0.51	>0.05
Degree of education	0.71	>0.05
Smoking	0.56	>0.05
Excessive drinking	0.61	>0.05
Domicile	0.24	>0.05

Note: ESR, erythrocyte sedimentation rate; RF, rheumatoid factor; IL-1, interleukin-1; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; CRP, c-reactive protein; BMI, body mass index; WBC, white blood cell; Hb, hemoglobin.

role in cellular apoptosis and proliferation. Moreover, a previous study has shown that, when secreted by macrophages, B-cells, T-cells, and IL-6 promotes macrophages to indirectly activate fibroblasts and release a large amount of vascular endothelial growth factor, leading to the formation of pannus and narrowing of joint space. Therefore, IL-6 has been considered an important factor in development of RA [19]. On the other hand, an important proinflammatory factor in development of inflammation, TNF-α levels in serum and joint fluid of RA patients have been reported in many studies [20, 21]. In addition, in another study, it was shown that secretion of TNF-α promotes IL-6 expression in synovial fibroblasts of RA patients [22]. In this current study, it was demonstrated that expression of IL-1, IL-6, and TNF-α in serum from RA patients was significantly higher compared to the control group, consistent with results presented by Hata et al. [23]. Thus, these results further prove that expression of IL-1, IL-6, and TNF-α may be used as diagnostic criteria for RA.

One of the most common indicators of inflammation, CRP effectively reflects the degree of inflammation in RA patients. In addition, an important indicator for diagnosis of RA, RF is of great significance for classification, treatment, and observation of RA. ESR is an index of routine clinical tests and plays a guiding role in dynamic observation of changes in the condition of RA patients. This present study determined levels of CRP, RF, and ESR and found that expression levels of these factors in the control group were significantly higher than in the observation group. Moreover, it was found that expression of D-D positively correlated to levels of IL-1, IL-6, TNF-α, CRP, RF, and ESR. Subgroup analysis showed that expression of D-D in subgroups, divided based on the DAS28 score, was significantly different with higher levels of D-D expression found in groups with higher levels of swelling joints and pain. Furthermore, ESR expression corresponded with levels of D-D expression, indicating that D-D is a more sensitive observation index for evaluating occurrence and development of RA.

This present study, however, has several limitations. The number of RA patients in this study was relatively small and D-D expression, during occurrence and development of RA, was not investigated in great detail. Therefore, future studies should have increased sample sizes more detailed investigation of D-D expression in RA.

In summary, D-D may be used as a non-specific inflammatory response index for clinical diagnosis of RA and may assist in guiding clinical treatment of RA.

Acknowledgements

This work was supported by the Weifang Science and Technology Development Project (2016YX042).

Disclosure of conflict of interest

None.

Address correspondence to: Jinmei Zhao, Department of Rheumatism and Immunity, Weifang People's Hospital, No.151 Guangwen Street, Kuiwen District, Weifang 261041, Shandong Province, China. Tel: +86-0536-8192130; E-mail: zhaojinmei25@163.com

References

[1] McInnes IB and Schett G. Pathogenetic insights from the treatment of rheumatoid arthritis. Lancet 2017; 389: 2328-2337.

[2] Zamora NV, Christensen R, Goel N, Klokker L, Lopez-Olivo MA, Kristensen LE, Carmona L, Strand V, Curtis JR and Suarez-Almazor ME. Critical outcomes in longitudinal observational studies and registries in patients with rheumatoid arthritis: an OMERACT special interest group report. J Rheumatol 2017; 44: 1894-1898.

[3] Smolen JS, Breedveld FC, Burmester GR, Bykerk V, Dougados M, Emery P, Kvien TK, Navarro-Compan MV, Oliver S, Schoels M, Scholte-Voshaar M, Stamm T, Stoffer M, Takeuchi T, Aletaha D, Andreu JL, Aringer M, Bergman M, Betteridge N, Bijlsma H, Burkhardt H, Cardiel M, Combe B, Durez P, Fonseca JE, Gibofsky A, Gomez-Reino JJ, Graninger W, Hannonen P, Haraoui B, Kouloumas M, Landewe R, Martin-Mola E, Nash P, Ostergaard M, Ostor A, Richards P, Sokka-Isler T, Thorne C, Tzioufas AG, van Vollenhoven R, de Wit M and van der Heijde D. Treating rheumatoid arthritis to target: 2014 update of the recommendations of an international task force. Ann Rheum Dis 2016; 75: 3-15.

[4] Chiu HY, Huang HL, Li CH, Chen HA, Yeh CL, Chiu SH, Lin WC, Cheng YP, Tsai TF and Ho SY. Increased risk of chronic kidney disease in rheumatoid arthritis associated with cardiovascular complications - a national population-based cohort study. PLoS One 2015; 10: e0136508.

[5] Heinlen L and Humphrey MB. Skeletal complications of rheumatoid arthritis. Osteoporos Int 2017; 28: 2801-2812.

[6] Okada Y, Wu D, Trynka G, Raj T, Terao C, Ikari K, Kochi Y, Ohmura K, Suzuki A, Yoshida S, Graham RR, Manoharan A, Ortmann W, Bhangale T, Denny JC, Carroll RJ, Eyler AE, Greenberg JD, Kremer JM, Pappas DA, Jiang L, Yin J, Ye L, Su DF, Yang J, Xie G, Keystone E, Westra HJ, Esko T, Metspalu A, Zhou X, Gupta N, Mirel D, Stahl EA, Diogo D, Cui J, Liao K, Guo MH, Myouzen K, Kawaguchi T, Coenen MJ, van Riel PL, van de Laar MA, Guchelaar HJ, Huizinga TW, Dieude P, Mariette X, Bridges SL Jr, Zhernakova A, Toes RE, Tak PP, Miceli-Richard C, Bang SY, Lee HS, Martin J, Gonzalez-Gay MA, Rodriguez-Rodriguez L, Rantapaa-Dahlqvist S, Arlestig L, Choi HK, Kamatani Y, Galan P, Lathrop M, Eyre S, Bowes J, Barton A, de Vries N, Moreland LW, Criswell LA, Karlson EW, Taniguchi A, Yamada R, Kubo M, Liu JS, Bae SC, Worthington J, Padyukov L, Klareskog L, Gregersen PK, Raychaudhuri S, Stranger BE, De Jager PL, Franke L, Visscher PM, Brown MA, Yamanaka H, Mimori T, Takahashi A, Xu H, Behrens TW, Siminovitch KA, Momohara S, Matsuda F, Yamamoto K and Plenge RM. Genetics of rheumatoid arthritis contributes to biology and drug discovery. Nature 2014; 506: 376-381.

[7] Glossop JR, Emes RD, Nixon NB, Haworth KE, Packham JC, Dawes PT, Fryer AA, Mattey DL and Farrell WE. Genome-wide DNA methylation profiling in rheumatoid arthritis identifies disease-associated methylation changes that are distinct to individual T- and B-lymphocyte populations. Epigenetics 2014; 9: 1228-1237.

[8] Abdollahi S, Koenders M, Joosten L, van de Loo F and van den Berg W. High efficacy of Toll-like receptor 4 targeting in murine and humanized models of rheumatoid arthritis in comparison with IL-1 and TNF inhibitors (THER6P.860). The Journal of Immunology 2014.

[9] Kondo Y, Kaneko Y, Sugiura H, Matsumoto S, Nishina N, Kuwana M, Jinzaki M and Takeuchi T. Pre-treatment interleukin-6 levels strongly affect bone erosion progression and repair detected by magnetic resonance imaging in rheumatoid arthritis patients. Rheumatology (Oxford) 2017; 56: 1089-1094.

[10] Kristensen AM, Stengaard-Pedersen K, Hetland ML, Horslev-Petersen K, Junker P, Ostergaard M, Hollsberg P, Deleuran B and Hvid M. Expression of soluble CD83 in plasma from early-stage rheumatoid arthritis patients is not modified by anti-TNF-alpha therapy. Cytokine 2017; 96: 1-7.

[11] Erlandsson M, Nadali M, Töyrä SS, Svensson MN, Jonsson IM, Andersson KM and Bokarewa MI. SAT0026 Signalling through insulin-like growth factor 1 receptor contributes to il-6 production and supports t cell dependent inflammation in rheumatoid arthritis. Ann Rheum Dis 2017; 76: 777.

[12] Gartner M, Alasti F, Supp G, Mandl P, Smolen JS and Aletaha D. Persistence of subclinical sonographic joint activity in rheumatoid arthritis in sustained clinical remission. Ann Rheum Dis 2015; 74: 2050-2053.

[13] Sparks JA, Chang SC, Nguyen UDT, Barbhaiya M, Tedeschi SK, Lu B, Costenbader KH, Zhang Y, Choi HK and Karlson EW. Smoking behavior changes in the early rheumatoid arthritis period and risk of mortality during thirty-six years of prospective followup. Arthritis Care Res (Hoboken) 2018; 70: 19-29.

[14] Moller B and Villiger PM. Inhibition of IL-1, IL-6, and TNF-alpha in immune-mediated inflammatory diseases. Springer Semin Immunopathol 2006; 27: 391-408.

[15] Folsom AR, Gottesman RF, Appiah D, Shahar E and Mosley TH. Plasma d-Dimer and incident ischemic stroke and coronary heart disease: the atherosclerosis risk in communities study. Stroke 2016; 47: 18-23.

[16] Yuan Y, Vora N, Sun CL, Li D, Soto-Perez-de-Celis E, Mortimer J, Luu TH, Somlo G, Waisman J, Smith D, Chao J, Katheria V, Synold T, Tran V, Mi S, Levi A, Arsenyan A, Choi J, Zavala L, Yost S and Hurria A. Association of pre-chemothera-

py peripheral blood pro-inflammatory and coagulation factors with reduced relative dose intensity in women with breast cancer. Breast Cancer Res 2017; 19: 101.

[17] Bashlakova NA, Tyabut TD, Buglova AE and Kundzer EV. AB0296 the coagulation state in female patients with rheumatoid arthritis and early atherosclerosis. Ann Rheum Dis 2015; 74: 992.

[18] Arkema EV, Lu B, Malspeis S, Karlson EW and Costenbader KH. Monocyte chemotactic protein-1 elevation prior to the onset of rheumatoid arthritis among women. Biomark Med 2015; 9: 723-729.

[19] Gualtierotti R, Ingegnoli F, Griffini S, Grovetti E, Meroni PL and Cugno M. Prothrombotic biomarkers in patients with rheumatoid arthritis: the beneficial effect of IL-6 receptor blockade. Clin Exp Rheumatol 2016; 34: 451-458.

[20] Li XY, Li H, Zhang Y, Gao S, Dong CP and Wu GF. Development of albumin coupled, cholesterol stabilized, lipid nanoemulsion of methotrexate, and TNF-alpha inhibitor for improved in vivo efficacy against rheumatoid arthritis. AAPS PharmSciTech 2017; 18: 2774-2782.

[21] Kutukculer N, Caglayan S and Aydogdu F. Study of pro-inflammatory (TNF-alpha, IL-1alpha, IL-6) and T-cell-derived (IL-2, IL-4) cytokines in plasma and synovial fluid of patients with juvenile chronic arthritis: correlations with clinical and laboratory parameters. Clin Rheumatol 1998; 17: 288-292.

[22] Pearson MJ, Herndler-Brandstetter D, Tariq MA, Nicholson TA, Philp AM, Smith HL, Davis ET, Jones SW and Lord JM. IL-6 secretion in osteoarthritis patients is mediated by chondrocyte-synovial fibroblast cross-talk and is enhanced by obesity. Sci Rep 2017; 7: 3451.

[23] Hata H, Sakaguchi N, Yoshitomi H, Iwakura Y, Sekikawa K, Azuma Y, Kanai C, Moriizumi E, Nomura T, Nakamura T and Sakaguchi S. Distinct contribution of IL-6, TNF-alpha, IL-1, and IL-10 to T cell-mediated spontaneous autoimmune arthritis in mice. J Clin Invest 2004; 114: 582-588.