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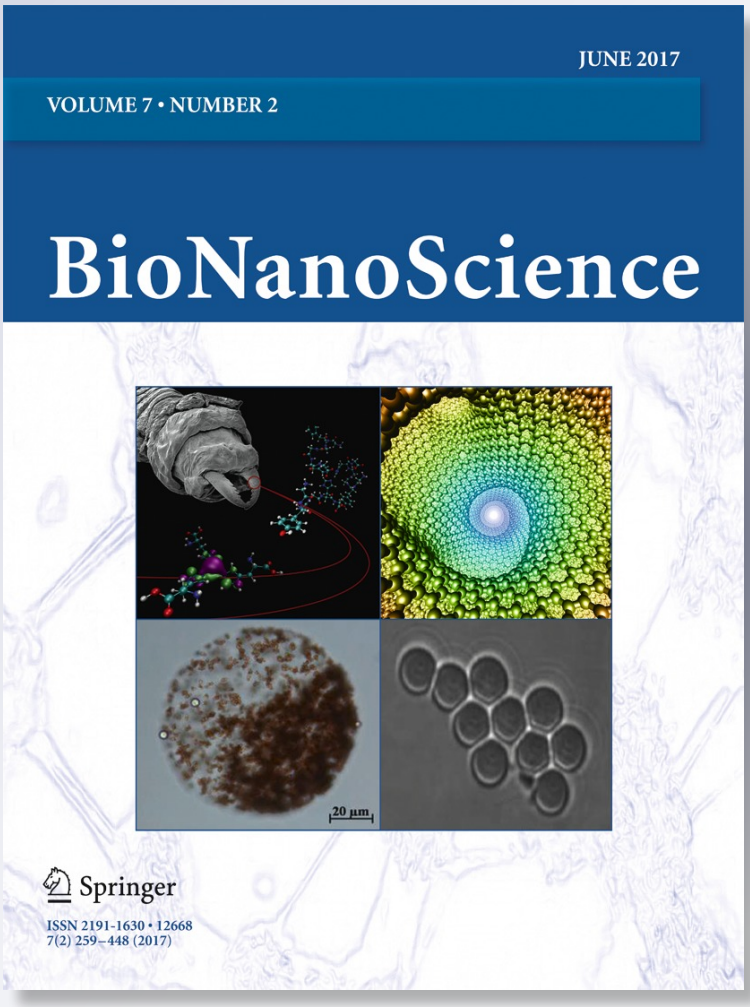

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Cytokine Levels in the Serum of Patients with Chronic Kidney Insufficiency Before and After Hemodialysis

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Abstract Chronic kidney insufficiency (CKI) is often the end point of a broad range of chronic kidney diseases and characterized with decreasing number of functionally active nephrons. Pathophysiological CKI is characterized by decreased glomerular filtration, which leads to accumulation of life-threatening toxic metabolites. Hemodialysis is the main therapeutic measure aimed to prolong patient's life until kidney transplant is available. The goal of this study is to analyze serum level of 21 cytokines in CKI. We have found that the serum level of several (IL-2Rα, IL-3, IL-12 (p40), IL-16, IL-18, HGF, MIF, CSF-1, MCP-3, CXCL12, SCF, IFN-α2, LIF, β-NGF, and CXCL1) cytokines and chemokines was upregulated in CKI without hemodialysis as compared to controls (p = 0.005). Interestingly, serum cytokines were also upregulated in serum of CKI patients who received hemodialysis. Upregulated cytokines are associated with inflammation and activation of Th1 lymphocytes. We suggest that hemodialysis has limited effect on serum cytokine levels. It could be concluded that therapeutic effect of hemodialysis is not associated with removal of inflammatory cytokines from circulation. Further studies will help better define the underlying cause of an increased inflammation in CKI and identify the laboratory criteria for anti-inflammatory therapy.

Keywords Chronic kidney disease · Hemodialysis · Inflammation · Serum · Cytokines

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1 Introduction

The worldwide frequency of CKI incidence is 8–16% with tendency to increase due to the growing elderly population [1]. There are two clinical laboratory tests used to monitor CKI which measure creatinine retention (coefficient of purification, measured by glomerular filtration) and changes in blood pH. CKI pathogenesis is based on the reduction of the number of glomeruli as well as decreased tubular function. There is no cure for CKI, and hemodialysis (HD) is used as a supportive measure until the kidney transplant is available.

It is believed that inflammation plays an important role in CKI pathogenesis. Therefore, it has been suggested that the ratio of serum pro- and anti-inflammatory cytokines can be used to determine the CKI progression [2]. Treatment of CKI may affect cytokine activation and impact development of the immune response and inflammation. Therefore, a study of cytokine activation in CKI will improve our understanding of the disease pathogenesis as well as help to understand the mechanism of the therapeutic effect of hemodialysis.

The goal of this investigation was to analyze the serum cytokine level of patients in CKI (Fig. 1) who received hemodialysis and those without.

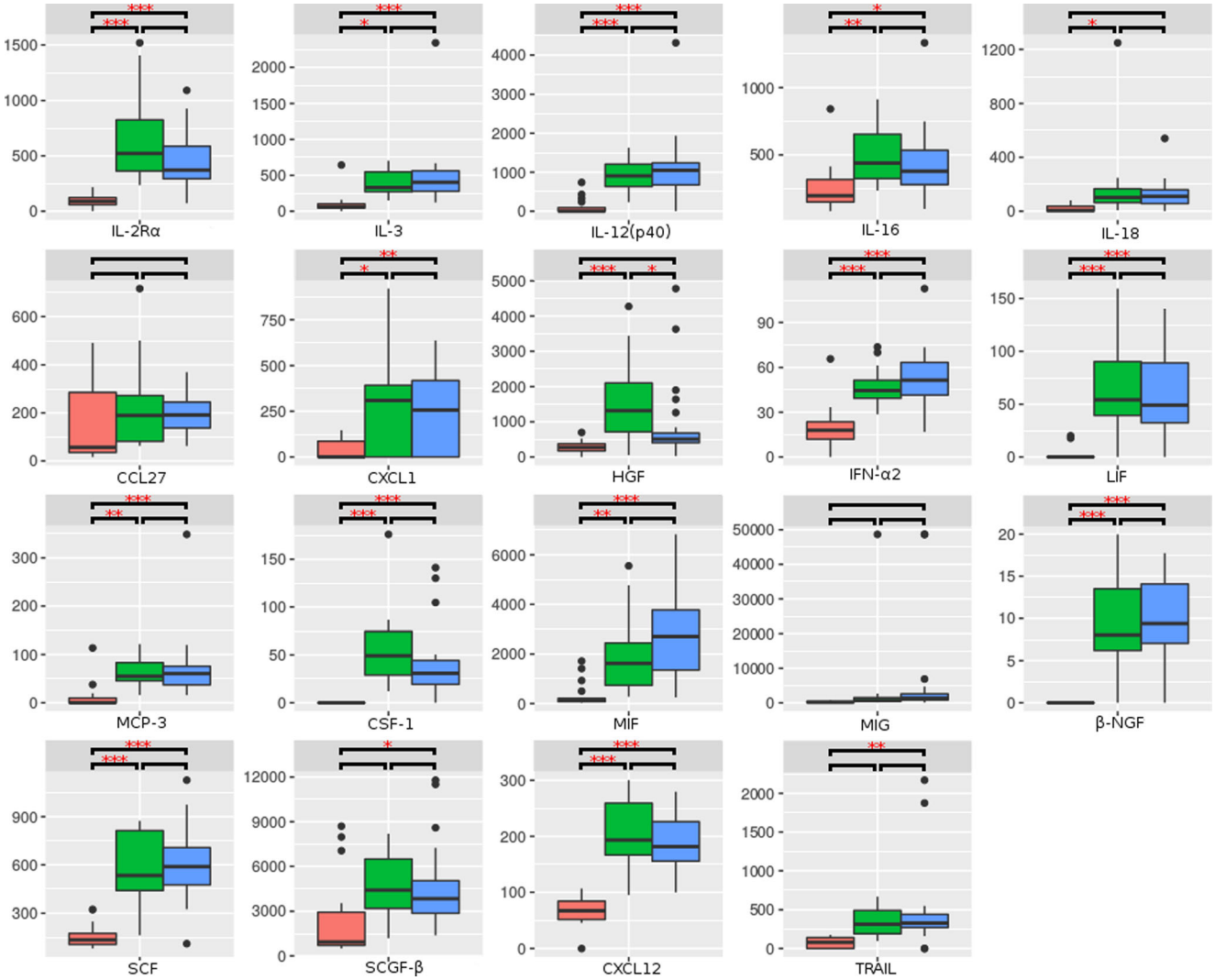

Fig. 1 Serum cytokine levels (pg/mL) in CKI. *Red box* indicates the control, *green box* indicates CKI without hemodialysis, *blue box* indicates CKI with hemodialysis, s*ingle asterisk* indicates p value < 0.05, *double asterisk* indicates p value < 0.01, and *triple asterisk* indicates p value < 0.001

2 Materials and Methods

Serum Serum samples were collected from CKI patients admitted to the Department of Kidney Transplantation of the Republican Clinical Hospital of the Ministry of Health, Republic of Tatarstan (Kazan, Russia).

Patients CKI patients were divided into two groups: (1) terminal stage CKI (28 cases; 14 men to 14 women), these patients were regularly receiving hemodialysis, and (2) CKI (31 cases; 15 men to 16 women), these patients were in predialysis stage. Patients received the dialysis three times a week (twice every other day) at the Department of Hemodialysis of the Republican Clinical Hospital. The diagnosis of CKI was based on the anamnesis, clinical observation, and laboratory data. Serum which was collected from 15 conditionally healthy donors (7 women and 8 men) presented was used as a control.

Multiplex Analysis Quantitative analysis of cytokines (IL-1α, IL-2Rα, IL-3, IL-12(p40), IL-16, IL-18, CCL27, CXCL1, MIG, MIF, colony stimulating factor-1 (CSF-1), mast cell proteinase-3 (MCP-3), CXCL12, SCF, stem cell growth factor beta (SCGF-β), hepatocyte growth factor (HGF), IFN-α2, leukemia inhibitory factor (LIF), β-NGF, TNF-β, TNF-related apoptosis-inducing ligand (TRAIL)) in blood serum was performed using a multiplex analyzer Bio-Plex200 System (BioRad) and kit Bio-Plex Pro™ Human Cytokine 21-plex Assay (BioRad), according to the manufacturer's recommendations.

Statistical Analysis Statistical analysis of the data obtained was performed in R environment (www.r-project.org). Correlation coefficients were evaluated using rcorr function (Hmisc). Statistically significant differences between the means of analyte levels in groups were accepted as $p < 0.05$

considering Bonferroni correction as a method of p value adjusting.

3 Results and Discussion

Level of 13 cytokines (IL-2Rα, IL-3, IL-12(p40), IL-16, MIF, M-CSF, MCP-3, CXCL12, SCF, IFN-α2, LIF, β-NGF, and CXCL1) was significantly increased in serum of CKI in both groups as compared to controls. Serum level of IL-1a and TNF-b was out of the detection range in all groups, so they were excluded from the analysis. Differences in the level of IL-18 and HGF were not significant between CKI patients who received dialysis compared to the control, but serum level of these cytokines was significantly higher in serum of CKI patients on pre-dialysis stage than that of the control. Serum level of CCL27 and MIG did not differ significantly between all groups.

Serum level of pro-inflammatory cytokine IL-12p40 was 35–40 times higher in all CKI patients when compared to controls. Additionally, serum IL-18 level was upregulated (five to tenfolds) in CKI patients on pre-dialysis stage compared to the control. Both IL-12p40 and IL-18 are produced by activated macrophages [3–5]. Therefore, it could be suggested that cytokines produced by activated macrophages contribute into the pathogenesis of CKI.

Interestingly, IL18 and IL12p-40 can activate Th1 lymphocytes [6]. IL-18 can stimulate synthesis of IFN-γ which is the key cytokine regulating Th1-type immune response. Increased serum levels of IFN-α and MCP-3 (3 and 13 times, respectively) were found in all patients with CKI. These cytokines are chemoattractant for monocytes mobilizing migration to the site of inflammation. Therefore, it could be proposed that CKI is characterized by upregulation of serum cytokines promoting Th1 lymphocytes activation and monocyte mobilization.

It is important to note that hemodialysis has limited effect on serum level of IL12p-40; nevertheless, hemodialysis decreased serum level of IL-18. Therefore, we suggest that anti-inflammatory effect of hemodialysis is limited on CKI pathogenesis.

Upregulation of IL-16 in the group with terminal stage of CKI (p = 0.0321) and pre-dialysis group (p = 0.0079) was detected. IL-16 (lymphocyte chemotaxis factor, LCF) is a chemoattractant for CD4+ T-lymphocytes, monocytes, and eosinophils [7]. Upon activation, IL-16 is released by endothelial cells, lymphocytes, macrophages, and eosinophils [8]. We have found that increased serum concentration of IL-16 positively correlates with upregulation of IL-2Rα in CKI patients (R = 0.46, p value < 0.001), which is in accordance with data published by Cruikshank et al. [9]. In both CKI patient groups, an increased serum level (three times) of CXCL1 was detected compared to the control. CXCL1 is constitutively expressed by stromal cells of bone marrow [10] and acts as a chemoattractant for lymphocytes and monocytes, as well as promotes B-cell proliferation.

Serum level of several growth factors, β-NGF, SCF, CSF-1, and HGF, was found upregulated in patients on pre-dialysis stage. β-NGF is a pleotropic factor, which is expressed by stromal cells as well as immune cells [11]. Also, SCF promotes proliferation, migration, survival, and differentiation of hematopoietic progenitor cells [12]. M-CSF stimulates differentiation of hematopoietic progenitor cells into mononuclear phagocytes [13]. Therefore, it could be suggested that CKI is characterized by proliferation of hematopoietic progenitors leading to increased monocyte/macrophage population.

HGF stimulates angiogenesis and proliferation of endothelial cells [14]. It has been shown that HGF is essential for regeneration of hepatic kidney and lung cells [15–17]. The HGF protective effect is linked to inhibition of the apoptosis and inflammation [18]. Increased serum level of IL-3 suggests activation of Th1 and Th2 cells and B-lymphocytes in CKI patients. IL-3 regulates growth and differentiation of hematopoietic cells [19]. Also, together with GM-CSF and M-CSF, IL-3 contributes into proliferation of myeloid progenitor cells [19].

4 Conclusions

In conclusion, analysis of serum cytokines in CKI suggests activation of macrophages and Th1 lymphocytes. Our data supports observation made by Lam et al. presenting data on high plasma level of IL-18, IL-6, and TNF-α in patients receiving hemodialysis [20]. We also have found increased serum level of growth and differentiation factors (β-NGF, SCF, M-CSF, and HGF) which may reflect growth and proliferation of hematopoietic stem cells.

It appears that the serum level of IL-18 and HGF is mostly affected by hemodialysis, while levels of 13 cytokines (IL-2Rα, IL-3, IL-12 (p40), IL-16, MIF, CSF-1, MCP-3, CXCL12, SCF, IFN-α2, LIF, β-NGF, and CXCL1) remained unchanged. Therefore, we suggest that therapeutic effect of hemodialysis may include downregulation of IL-18 and HGF serum level in CKI patients.

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