Immune system dysfunction and malnutrition in hemodialysis patients part I – risk factors

Dr. Barbara-Joanna Bałan *(Medical University of Warsaw, Department of Immunology, Biochemistry and Nutrition)*

Mrs. Anna Kaczorek *(Medical University of Warsaw, Nutrition)*

Dr. Marta Stelmasiak *(Kazimierz Pulaski University of Technology and Humanities in Radom, Faculty of Medical and Health Sciences)*

Abstract

The main causes of death in hemodialysis patients (HD) are cardiovascular diseases (CVD) and infections both of which are linked to impaired immune function. In patients with end stage renal disease, immunodeficiency and immune activation co-exist. Changes in the immune system are complex, but to some extent result from malnutrition or insufficiency in essential vitamins or trace elements. Based on the link between high mortality, immune system dysregulation, vitamins and trace elements insufficiency, it emphasizes the potential role of nutritional counseling and supplementation.

Introduction

Immune function dysregulation and malnutrition in hemodialysis patients (HD) are factors that contribute to the high prevalence of cardiovascular disease (CVD) and infections, which are the major cause of death in this group of patients. A better understanding of the complex mechanisms and correlations existing between immune dysfunction and nutritional status may contribute to the engagement of nutritional supplementation, which may modulate some immune functions.

Immune system alteration in chronic kidney disease (CKD) involves innate and adaptive systems [1]. On the one hand patients undergo immune activation, which leads to atherosclerosis, anorexia, insulin resistance and an increase of muscle metabolism. On the other hand CKD is related to immune suppression, which leads to an increase in infections and poorer response to vaccination [2]. It is thought that the major causes of immune deficiency are uremic toxins, whereas chronic inflammation arises due to dialysis procedure itself. However, despite extensive studies, the precise causes of immune dysregulation in end stage renal disease (ESRD) patients are not known [2]. Abnormalities in leukocyte function are presented in table 1.

Table 1. Leukocyte abnormalities observed in ESRD patients

Cells	Suppression	Activation
Monocytes	- downregulation of opsonin receptors - poor antigen presentation - poor antibody response to vaccines - impaired chemotaxis, adhesion, migration, phagocytosis and bactericidal activities	- increased level of neopterin - increased expression of CD14 - imbalance between CD14/CD16 subsets - overexpression of adhesion molecules - increased production of cytokines and cytokine inhibitors
Neutrophils	- downregulation of opsonin receptors - impaired chemotaxis, adhesion, migration, phagocytosis and bactericidal activities	- increased generation of radical oxygen species (ROS) and chlorinated oxidants via NADPH oxidase and myeloperoxidase - increased liberation of proteases - upregulation of TLR-4 expression with increased level of IL-6 and TNF-α [3]
Dendritic cells	- downregulation of TLR-2 and TLR-4 expression on cell surface and disturbances in their proportion lead to higher susceptibility to bacterial infection [4]; - impaired maturation and decreased endocytosis [5]	- increased LPS (lipopolysaccharide) stimulated production of TNF-α [5]
Natural Killer	- lower NK cells (CD3-,CD16+) count in peripheral blood [6]	
T Lymphocytes	- anergy and poor delayed-type hypersensitivity - defective cytotoxic effect towards intracellular pathogens and viruses - defective proliferation - defective production of IL-2 and IFN-γ - imbalance between Th1/Th2 cytokines - poor antibody responses to vaccines - decreased of Invariant natural killer T (/NKT) [7] - decreased percentage of NKL T-cell (natural killer-like T-cell) and zeta-chain expression (responsible for signaling pathway in cells) in HD [8]	- increased IL-2 R expression and release - increased early activation markers - increased rate of apoptosis - increased apoptosis and reduction in regulatory T-cells (CD4+/CD25+bright+FoxP3+) [3]
B Lymphocytes	- defective production of opsonins (IgG and IgM) - poor antibody response to vaccines	- increased incidence of autoantibodies - increased CD23 expression and release - enhanced apoptosis [9]

Descamps-Latscha, Jungers P., Witko-Sarsat V., Immune System Dysregulation in Uremia: Role of Oxidative Stress, Blood Purification, 2002, 20, str. 481-484 [2] in modification

Innate immune system

The innate immune system is evolutionary older than adaptive immune system, and it is the immediate and universal mechanism of host defence against pathogens [1].

Although it does not create long-lasting immunological memory of antigens it is capable of eliminating infections. Innate immunity cells recognise pathogen with pattern recognition receptors [10]. Among others, one of the most important are Toll-like receptors (TLR); disturbances in their expression and activity may lead to an increased susceptibility to infection and to the development of systemic inflammation [11].

Studies have shown that monocyte and monocyte-derived dendritic cells in ESRD patients and in uremic milieu are characterised by decreased endocytosis and diminished maturation [1]. It has also been reported that non-dialysed patients with ESRD have an increased level of the CD14+CD16+ monocyte subset, which is linked to an increase in production of inflammatory cytokines, radical oxygen species and up-regulation of TLR-2 and TLR-4 expression, which predisposes to a higher risk of inflammation [3]. Furthermore, an increase of CD14+CD16+ monocytes is linked to a greater risk of a cardiovascular event [12]. However, some findings suggest that in ESRD patients there is a reduction in TL-4 expression, due to the chronic impact of endotoxin and reduction of TLR-2 expression on circulating monocytes [13]. Lorenzen *et al*. found that there is no link between CD14+/TLR-4+ monocytes and cardiovascular events and cardiovascular death in HD patients [11].

The impaired function of macrophages may have an effect on uremic toxins, e.g. phenylacetic acid, an inhibitor of inducible nitric oxide synthase (iNOS), which plays a crucial role in synthesis of nitric oxide - an important mediator of macrophages cytotoxity [14].

ESRD patients exhibit an impairment of neutrophil functions. Dialysis procedures with bio-incompatibility materials lead to polymorphonuclear neutrophils (PMN) activation [15]. On the other hand their bactericidal capabilities are 20% weaker than those from healthy subjects. To some extent dialysis improves this capability, but after dialysis antimicrobial capability remains 10% weaker than in healthy controls. It has also been shown that there is no difference in the percentage of neutrophils producing ROS and capable of phagocytosis, between ESRD patients and healthy controls, which is contradictory to many other studies. The underlying cause of alternations in PMN is not well understood, but it is suspected that they might be influenced by anemia, malnutrition and zinc insufficiency [16]. Apoptosis of neutrophils in ESRD patients remains controversial. Some authors indicate that there is accelerated apoptosis of neutrophils in dialysis patients due to uremic retention solutes. Neutrophils with delayed apoptosis are prone to undergo necrosis, leading to the release of cytotoxic metabolites and pro-

inflammatory substances. Other studies have shown activation of apoptosis stimulated by proapoptotic agents, such as advanced glycation end products (AGE), TNF-α and oxidized LDL [1].

Other impairments of the innate immune system include depletion of dendritic cells from ESRD patients, especially in the plasmacytoid subset, what is exacerbated by hemodialysis [5]. Studies conducted to identify NK (natural killer) alternations and dysfunction in HD patients led to conflicting reports. Vacher-Coponat *et.al.* showed that NK cells in HD patients are decreased rather than have impaired function [9]. Uremia affects also mast-cells by inducing their proliferation and increasing their density [17], which is favorable for atherogenesis.

Adaptive immune system

The role of the adaptive immune system is to create a memory of immunological response. The highly-specialized cells involved in this process are lymphocytes B and T [10].

Many authors have noted a variety of disorders in acquired immunity. In dialysis patients there is a decrease in lymphocyte count and a reduction in the CD4/CD8 ratio due to apoptosis, impairment of activation and differentation of T cells into effector memory T cells. These impairments are related to oxidative stress, inflammation and iron overload [3]. Increased apoptosis of lymphocytes may also be an effect of bio-incompatibility and permeability of the dialysis membrane [18].

The characteristic impairment of T cell activation in dialysis patients is an increase in Th1 cell level while Th2 cell levels remain normal, which correlates with a higher percentage of monocytes capable of secreting IL-12 [2], an important component of atherosclerosis development [19]. Decreased activation of T helper lymphocytes in ESRD patients is caused by alterations in antigen presenting cells. Responsible for this is a reduction of CD86 expression on monocytes, which is an important stimulator for T helper cell activation [20]. Uremic milieu also decreases the number of TCR/CD3 receptors, which take part in antigen recognition and T cell activation [1].

Alterations in humoral immunity in ESRD are not as considerable as abnormalities in the cellular response. Adults and children with ESRD exhibit B lymphopenia. ESRD children, especially dialyzed, have shown to have diminished levels of CD27+ memory B cells, which may cause a decrease in production of IgM and IgD and can increase the risk of infection. Uremic milieu might be a cause for lymphopenia,

because it increases the susceptibility of B cells to apoptosis and may lead to the resistance of transitional B cells to differentiation and survivals signals [3].

Inflammation and oxidative stress

Among dialysis patients about 35-65% develop chronic inflammation [21]. Chronic inflammation triggers development of CVD, which is the most common cause of death in this group of patients. Chronic inflammation affects blood lipids, vascular endothelium and plasma proteins in a manner that contributes to vascular damage [22]. Understanding the mechanisms leading to it and treatment methods may reduce mortality levels in this group of patients.

Biomarkers of inflammation are elevated levels of acute phase proteins (CRP, amyloid, fibrinogen and ceruloplasmin). The increase of synthesis of proinflammatory cytokines: IL-1 (IL-1β), IL-6 and TNF-α has a stimulatory effect on higher production of proinflammatory proteins [21]. In addition acute phase proteins include also adhesion molecules: VCAM-1 and I-CAM-1, the expression of which can be induced by CRP. Specifically, IL-6 activates the endothelium, stimulates release of adhesion molecules, causes increase of platelet adhesion, stimulates hyperplasia of smooth muscle cells and intensifies thrombotic processes. TNF-α is a mediator of endothelial activation and is involved in vascular cell calcification [21]. In addition, IL-6 and TNF-α inhibit the production of lipoprotein lipase in adipocytes, leading to increased lipolysis and dyslipidemia. Cytokines can also cause anemia, which is an important factor in accelerating the development of atherosclerosis [23].

Whereas the level of some acute-phase proteins is elevated, the level of some important proteins as albumin, prealbumin, transferrin, IGF-I and retinol binding protein is low, serving as a marker of malnutrition [8]. With regards to the loss of structural proteins connected to maintenance inflammation, there is an elevated risk of development of systemic inflammatory response syndrome (SIRS) and compensatory anti-inflammatory response syndrome (CARS).

Inflammation in ESRD patients may be caused by uremia, decreased clearance of cytokines, underlying disease, bacterial and viral infections and coexisting diseases such as lung disease, heart failure and fluid overload [23]. One of the reasons for development of inflammation is the presence of oxidative stress [21, 8]. Increased production of reactive oxygen species is also one of the main causes of endothelial dysfunction. Uremia is characterized by an accumulation of proteins with changed structure and function due

to oxidation, glycation and carbonylation. Increased levels of lipid and protein oxidation markers in HD patients correlates positively with CRP levels [8] and may affect the development of inflammation and atherosclerosis, as shown in Table 2.

Table 2. Altered structure proteins and their impact on the immune system

Process	Product	Effect on the immune system
Glycation	AGE	- in vitro modulate PMNL chemotaxis and accelerate apoptotic cell death, what may lead to diminished immune function [24]; - activation of macrophages, oxidative stress, angiogenesis and cell proliferation [24].
Oxidation	AOPPs (advanced oxidation protein products)	- in vitro ability to activate the respiratory burst in monocytes and increase production of TNF [2]; - activate induction and apoptosis of macrophages stronger than ox-LDL [2].
Oxidation	ox-LDL	- toxic effect on endothelium, increase migration and adhesion of monocytes to endothelial cells, increase peroxidation of lipoproteins [25]; - stimulate VCAM-1 expression [19]; - reduction and apoptosis of regulatory lymphocytes $CD4^+CD25^+$ [3].

Of particular importance in ESRD patients is the impairment of high-density lipoprotein (HDL), level of which decreases with the progress of renal failure [22]. HDL is involved in the metabolism of lipoproteins rich in triglycerides, has antioxidant properties and inhibits the cytokine-induced platelet adhesion to endothelial cells and oxidation of LDL [26]. HDL level decreased due to an increase in serum of amyloid synthesis during inflammation and a reduction in the level of Apo AI (the main protein part of the HDL). SAA replacing Apo AI in HDL, impaires antioxidant capability and may cause the opposite proinflammatory effect of HDL [22].

Production of nitric oxide (NO) in patients with CKD is diminished. The reason for this impairment may be increased level of ROS, decreased level of tetrahydrobiopterin (BH4), increased level of NO synthase inhibitors as ADMA (asymmetric dimethylarginine) or decreased level of L-arginine [8]. Various studies show conflicting results on the level of L-arginine in plasma in CKD patients [27]. A normal level of NO has a positive effect on the smooth muscle of vessels, inhibitnig their proliferation and has anti-inflammatory properties. Diminished NO production enhance the risk of atherosclerosis and hypertension [19].

Immune function disregulation caused by malnutrition

For many years several authors raised the issue of the impact of malnutrition on the immune system dysfunction. Along with a reduction in BMI and decreased level of albumin, the risk of death from a cardiovascular event increased [22]. Additionaly, Lie et al. have shown an inverse relationship between cholesterol level and total mortality ratio and a U-shaped relationship with mortality from cardiovascular disease in the presence of both malnutrition and inflammation [8].

In dialysis patients, protein-calorie malnutrition is accompanied by a decrease in circulating lymphocytes and impairment of their function. Malnourished patients also have decreased levels of IgA, IgG and IgM [1]. Sayalioglu et al. showed that level of albumin, cholesterol and triglycerides in patients undergoing hemodialysis, correlates positively with the total number of lymphocytes of each subpopulation [28].

At the same time, the deficiency in certain vitamins and minerals can contribute to the immune system dysregulation, shown in Table 3.

Table 3. Immune system dysregulation resulting from deficiency of vitamins and minerals

Vitamins and minerals	**Immune system dysregulation**
↓Vitamin D	- low level of IL-10, high level of sIL-R2 [29].
↓Zinc	- impaired proliferation and function of T lymphocytes, monocytes, NK cells and other immunocompetent cells, impaired production of cytokines: IL-2, IL-6, TNF-α, INF-γ [30]; - stimulation of apoptosis in cultured cells [30]; - ↓ activity of superoxide dismutase [31].
↓Iron	- impaired lymphocytes proliferation [32).
↓Selenium	- impairment of glutathion peroxidase activity (GPx) in plasma, red blood cells and peripheral blood neutrophils [33]; - ↓ glutathione peroxidase [31].

Characteristic of dialysis patients are lower levels of selenium and zinc and a higher level of copper than in healthy controls. Guo et al. observed a significant relationship between the number of CD3 and zinc, selenium and iron as well as between CD4 and zinc and selenium [32].

Malnutrition may also lead to development of oxidative stress. CKD patients with malnutrition before dialysis have an increased biochemical signs of oxidative stress compared with patients with normal nutritional status [21]. Oxidative stress might be an effect of important components insufficiency, which are involved in the mechanism of defence against ROS: transferrin, albumin, vitamin C, vitamin E in cell membranes of erythrocytes and neutrophils and deficiency of selenium and reduced glutathione [34].

Results of various studies in the field of immune system dysfunction in CKD patients indicate a variety of disorders that cause both immunodeficiency and chronic inflammation. Furthermore, the research results are contradictory to each other, highlighting the need for further research in this field. The existing link between malnutrition and immune system dysregulation, suggests that immunonutrition might improve the immunological status in this group of patients.

Competing Interests statement

The authors declare no conflict of interest.

References:

1. Kato S, Chmielewski M, Honda H, et al.: Aspects of Immune Dysfunction in End-stage Renal Disease, Clin J Am Soc Nephrol, 2008: 1526-1533
2. Descamps-Latscha B, Jungers P, Witko-Sarsat V: Immune System Dysregulation in Uremia: Role of Oxidative Stress, Blood Purif, 2002, 20: 481-484
3. Vaziri ND, Pahl MV, Crum A, Norris K: Effect of uremia on structure and function of immune system, J Ren Nutr, 2012, 22, 1: 149-156
4. Kazimierczak K, Kopeć W, Klinger M: Receptory Toll-podobne (TLR) w patogenezie chorób nerek, Pol Merkuriusz Lek, 2007, XXIII, 137: 382-385
5. Agrawal S, Gollapudi P, Elahimer R, et al.: Effects of end-stage renal disease and haemodialysis on dendritic cell subsets and basal and LPS-stimulated cytokine production, Nephrol Dial Transplant, 2009, 25: 737-746
6. Liszka M, Żukowska-Szczechowska E, Grzeszczak W, et al.: Natural killer cell count in hemodialysis patients, Pol Arch Med Wew, 1998, 100, 1: 9-18
7. Peukert K, Wingender G, Patecki M, et al.: Invariant natural killer T cells are depleted in renal impairment and recover after kidney transplantation, Nephrol Dial Transplant, 2014, 1020-1028
8. Bonanni A, Mannucci I, Verzola D, et al.: Protein-Energy Wasting and Mortality in Chronic Kidney Disease, Int J Environ Res Public Health, 2011, 8: 1631-1654
9. Vacher-Coponat H, Brunet C, Lyonnet L, et al.: Natural killer cell alterations correlate with loss of renal function and dialysis duration in uraemic patients, Nephrol Dial Transplant., 2008, 23 (4): 1406-1414
10. Kędziora S, Słotwiński R: Molekularne mechanizmy towarzyszące rozpoznawaniu patogenu przez receptory wrodzonej odporności, Postepy Hig Med Dosw (Online), 2009, 63: 30-38
11. Lorenzen JM, David1 S, Richter A, et al.: TLR-4+ peripheral blood monocytes and cardiovascular events in patients with chronic kidney disease—a prospective follow-up study, Nephrol Dial Transplant. 2011, 26 (4): 1421-1424
12. Rogacev KS, Seiler S, Zawada AM, et al.: CD14++CD16+ monocytes and cardiovascular outcome in patients with chronic kidney disease, Eur Heart J., 2011, 32, 1: 84-92

13. Koc M, Toprak A, Arikan H, et al.: Toll-like receptor expression in monocytes in patients with chronic kidney disease and haemodialysis: relations with inflammation, Nephrol Dial Transplant, 2011, 26: 955-963
14. Schmidt S, Westhoff T, Krauser P, et al.: The ureamic toxin phenylacetic acid impairs macrophage function, Nephrol Dial Transplant, 2008, 23, 11: 3485-3493
15. Costa E, Rocha S, Rocha-Pereira P, et al.: Neutrophil Activation and Resistance to Recombinant Human Erythropoietin Therapy in Hemodialysis Patients, Am J Nephrol, 2008, 28: 935-940
16. Anding K, Gross P, Rost JM, et al.: The influence of uraemia and haemodialysis on neutrophil phagocytosis and antimicrobial killing, Nephrol Dial Transplant, 2003, 18,10: 2067-2073
17. Swaminathan S, Shah S: Novel inflammatory mechanisms of accelerated atherosclerosis in kidney disease, Kidney Int, 2011, 80: 453-463
18. Soriano S, Martin-Malo A, Carracedo J, et al.: Lymphocyte Apoptosis: Role of Uremia and Permeability of Dialysis Membrane, Nephron Clin Pract, 2005, 100: c71-c77
19. Undas A, Szczeklik A: Miażdżyca, in: Interna Szczeklika, Gajewski P, Kraków 2013, 160-164
20. Girndt M, Sester U, Kaul H, et al.: Impaired cellular immune function in patients with end-stage renal failure, Nephrol Dial Transplant, 1999, 14: 2807-2810
21. Jakuszewski P: Zespół MIA (malnutrition, inflammation, atheosclerosis) u chorych z przewlekłą terminalną niewydolnością nerek leczonych nerkozastępczo, Nefrol Dial Pol, 2005, 9: 156-159
22. Kaysen GA: Association between Inflammation and Malnutrition as Risk Factors of Cardiovascular Disease, Blood Purif, 2006; 24: 51-55
23. Czekalski S, Pawlaczyk K, Oko A: Rozwój zespołu niedożywienie - zapalenie - miażdżyca (zespół MIA) u chorych z upośledzeniem czynności nerek leczonych zachowawczo, Nefrol Dial Pol, 2004, 8, 2: 12-115
24. Cohen G, Rudnicki M, Walter F, et al.: Glucose-Modified Proteins Modulate Essential Functions and Apoptosis of Polymorphonuclear Leukocytes, J Am Soc Nephrol, 2001, 12, 6: 1264-1271
25. Steciwko A, Mastalerz-Migas A: Przewlekła choroba nerek i jej wpływ na choroby serca i naczyń, Terapia, 2006, 14, 9: 77-80
26. Vaziri ND: Dyslipidemia of chronic renal failure: the nature, mechanisms, and potential consequences, Am J Physiol, 2006, 290: 262-272
27. Rysz J, Guga P, Grycewicz T, et al.: Blood serum and neutrophil L-arginine concetrations and nitric oxide release by neutrophils in chronic uremic patients and healthy persons, Med Sci Monit, 2003, 9, 7: 311-315
28. Sayarlioglu H, Erkoc R, Demir C, et al.: Nutritional Status and Immune Functions on Maintenance Hemodialysis Patients, Mediators Inflamm, 2006: 1-4
29. Youssef DM, Elshal AS, Abo Elazem AA: Assessment of Immune Status in Relation to Vitamin D Levels in Children on Regular Hemodialysis, Saudi J Kidney Dis Transpl, 2012: 267-273
30. Weissgarten J, Berman S, Bilchinsky R, et al.: Total Cell-Associated Zn++ and Cu++ and Proliferative Responsiveness of Peripheral Blood Mononuclear Cells From Patients on Chronic Hemodialysis, Metabolism, 50, 3, 2001: 270-276

31. Pietrzak I, Bladek K, Bulikowski W: Aktywność układu antyoksydacyjnego a zawartość cynku, miedzi i selenu we krwi u chorych w okresie schyłkowej niewydolności nerek leczonych powtarzaną hemodializą, Biul Magnezol, 2001, 6, 3: 331-339
32. Guo CH, Wang CL, Chen PC, et al.: Linkage of trace elements, peripheral blood lymphocytes, inflammation, and oxidative stress in patients undergoing either hemodialysis or peritoneal dialysis, Perit Dial Int, 2011, 31, 5: 583-591
33. Pędzik A, Paradowski M, Rysz J: Stres oksydacyjny w nefrologii, Pol Merkuriusz Lek, 2010, XXVIII, 163: 56-60
34. Locatelli F, Canaud B, Eckardt KU, et al.: Oxidative stress in end-stage renal disease: an emerging threat to patient outcome, Nephrol Dial Transplant, 2003, 18: 1272-1280