Evaluation of renal function and immune system cells in elderly individuals from São Paulo City

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OBJECTIVES: Both renal function and immune system function decline with age. Although controversial, a significant number of studies have shown that the decline in kidney function is associated with the worsening of the immune system. These findings are reinforced by the increased susceptibility to infections and deficient immunization coverage after vaccination both in patients with chronic renal disease and in elderly individuals. Our objective was to evaluate a non-institutionalized elderly population from São Paulo City and correlate the estimated glomerular filtration rate with the percentage of lymphocytes in circulation.

METHODS: A random population of 237 individuals (107 men and 130 women), ranging in age from 60 to 101 years, who were enrolled in the Health, Well-Being and Aging Study was evaluated for renal function (Modification on Diet in Renal Disease formula) and lymphocyte percentage (flow cytometry).

RESULTS: Aging was associated with a decrease in the estimated glomerular filtration rate in both male and female individuals. We did not identify a significant correlation between the estimated glomerular filtration rate and either the percentage of CD4, CD8, and B cells or CD4/CD8 ratio. The median percentage of CD8+ T cells was significantly lower in individuals with an estimated glomerular filtration rate $\geq$60 mL/min/1.73 m^2.

CONCLUSIONS: In this study, no statistical correlation was found between the estimated glomerular filtration rate and either the lymphocyte phenotype (CD4+, CD8+, and CD19+ cells) or the CD4/CD8 ratio in blood.

KEYWORDS: Renal Function; Elderly; Immune System; Flow Cytometry.

◼ INTRODUCTION

Renal function declines with age (1) and corresponds to a high prevalence of chronic kidney disease in older individuals (2). Additionally, the percentage and function of immune cells have been reported to change in elderly individuals (3).

Teixeira et al. (4) evaluated individuals ranging in age from 60 to 101 years and observed that the percentages of T (CD4+) and B (CD19+) cells were decreased, whereas there was an increase in the percentage of T CD8+ cells. The study also revealed a decrease in the CD4/CD8 ratio, and all changes were more significant in men than in women.

A significant number of studies have shown that the decline in kidney function is associated with changes in the immune system. However, these studies are controversial, probably because of the different parameters used to measure kidney function and immune response (5).

In healthy elderly individuals, the decreased cellularity in the peripheral blood has been attributed to thymus involution (6) rather than bone marrow insufficiency (7). However, in patients with chronic kidney disease, other factors, such as malnutrition, parathyroid hormone deficiencies, and trace element deficiencies, could contribute to the decrease in the cell numbers observed in the blood (8).

Verkade et al. and Litjens et al. (9,10) showed that the progressive loss of kidney function is associated with poor generation of antigen-specific T cells after vaccination and increased susceptibility to infection. Betjes et al. (11) evaluated young (25-45 years old) individuals, either healthy or with chronic kidney disease, and observed that changes in renal function were associated with significant decreases in circulating naïve CD4+, naïve CD8+, and memory CD4+ cells and discrete changes in memory CD8+ T cells. The percentages of CD4+ and CD8+ T cells in young individuals with chronic kidney disease were similar to those observed in healthy elderly individuals (60-80 years old), suggesting that the decrease in kidney

function is associated with premature immunological aging. Guo et al. (8) showed that the CD4/CD8 ratio was significantly lower in patients with chronic kidney disease compared with healthy individuals.

Libetta et al. (12) observed that patients with chronic kidney disease exhibited significantly lower peripheral blood cell secretion of IFN-γ and IL-12 compared with healthy individuals. In contrast, these patients secreted constitutively more IL-4 and IL-10 than healthy individuals. Thus, it was suggested that these patients exhibit a deviation in their immune system that appears to correspond to the existence of a cell phenotype resulting in strong chronic activation of the immune system that inhibits a further response to acute stimulation, i.e., vaccination and new infections.

B cells, responsible for antibody production, are generated and matured in bone marrow mostly by the action of IL-7 (13,14). A decrease in B cells has been shown in children (15) and adults (16) with chronic kidney disease and could be the reason for the defective humoral (antigen-specific antibody production) response to infections, vaccinations and recall antigens in these patients.

The elderly Brazilian population is increasing, and there are few reports on the condition of their health. Therefore, our aim was to evaluate renal function in a random elderly (60 to 101 years old) population from São Paulo City and to correlate renal function with the percentage of T (CD4+ and CD8+) and B (CD19+) cells in the blood.

■ METHODS

The present study is part of a larger epidemiologic survey called the Health, Well-Being and Aging Study, which was coordinated by the Pan-American Health Organization, Washington, and conducted in Brazil by the School of Public Health of the University of São Paulo. From 2000 to 2001, the Health, Well-Being and Aging Study evaluated a sample of 2,143 non-institutionalized individuals, representing 836,204 aging people (60 years and older) living in the municipality of São Paulo, who were selected through multi-stage sampling. In 2006, the School of Public Health continued the survey in São Paulo and transformed it into a multi-cohort study with 1,115 individuals from the previous study who agreed to participate in the follow-up.

Trained examiners visited the participants at home and gathered information regarding socioeconomic variables, general health and living conditions. They also evaluated anthropometric and physical parameters and collected blood.

In the present study, the same inclusion/exclusion criteria were applied as the criteria cited above, and the individuals were enrolled as their biological samples were received. After obtaining written informed consent, the creatinine and blood lymphocyte levels of a total of 237 individuals (107 men and 130 women), between the ages of 60 and 101 years, were evaluated. The study was approved by the Ethics Committee of the University of São Paulo (USP), School of Public Health, Protocol number 2044/10.

A 3-mL sample of blood in EDTA was collected from each individual to evaluate serum creatinine and immune cell levels.

Creatinine measurement: Serum creatinine (SCr) levels in the blood were measured with a Dimension RXL (Siemens Laboratory Diagnostics, Tarrytown, NY, USA) automatic analyzer.

Immune cell phenotyping: A total of 100 μL of blood was lysed with Tris-buffered solution for 10 minutes and centrifuged at 377 g for 5 minutes. The cells were washed in PBS twice and centrifuged at 377 g for 5 minutes. The cells were then incubated with monoclonal antibodies (CD3PerCP, CD4FITC, CD8Pe-tritest, and CD19Pe; BD Biosciences, San Jose, California) to determine the percentages of T and B lymphocytes using flow cytometry in a FACSCalibur cell counter (BD Biosciences).

Estimated glomerular filtration rate (eGFR): SCr was used to calculate the eGFR according to the Modification of Diet in Renal Disease (MDRD) formula: eGFR (mL/min/1.73 m^2) = $186 \times (S_{cr})^{-1.154} \times (age)^{-0.208} \times 0.742$ (if female).

Statistical analysis

Variables were analyzed using Pearson's correlation test, and p-values<0.05 were considered to indicate statistical significance. Individuals were evaluated according to whether they had an eGFR lower or higher than 60 mL/min/1.73 m^2 using the Student's t-test. The Minitab software version 16.1 was used for all statistical tests.

■ RESULTS

Table 1 shows the clinical characteristics of the 237 elderly individuals studied. In total, 144 individuals had a serum creatinine level higher than 0.9 mg/dL, 66 individuals had glucose levels ≥100 mg/dL, and one individual had an albumin level higher than 5.0 g/dL.

Renal function evaluation

The use of serum creatinine as a method to evaluate renal function presents limitations that are even more pronounced in the elderly due to their decreased muscle mass, nutritional status, use of multiple drugs, and comorbidities. For example, a marked reduction in GFR can occur before the serum creatinine rises (it is possible for up to 50% of kidney function to be lost before the creatinine level changes).

Several equations (the Modification of Diet in Renal Disease (MDRD), Cockroft-Gault (CG), and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations) were developed to estimate the eGFR. However, because all of these equations use serum creatinine, they lack complete accuracy. To better evaluate renal function in our studied individuals, we calculated the eGFR using the Modification of Diet in Renal Disease (MDRD) equation.

Figure 1A shows a negative statistical correlation between eGFR and age, indicating that kidney function (i.e., eGFR) decreases with age. When eGFR was evaluated according to gender, this correlation was still observed (Figure 1B).

We recently published results showing that T and B cell levels change in elderly individuals, which could account for the decreased immune response observed in this

Table 1 - Baseline clinical characteristics of the 237 elderly individuals.

Gender	Female (n = 130) Male (n = 107)
Age	79.1 ± 9.6 (60-101)
Glucose (mg/dL)	95.0 ± 34.4 (32-373)
Albumin (g/dL)	3.79 ± 0.36 (2.8-5.7)
Creatinine (mg/dL)	1.03 ± 0.35 (0.4-3.37)
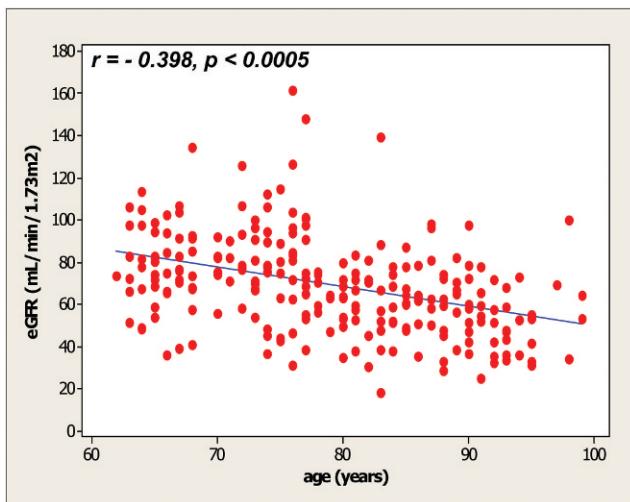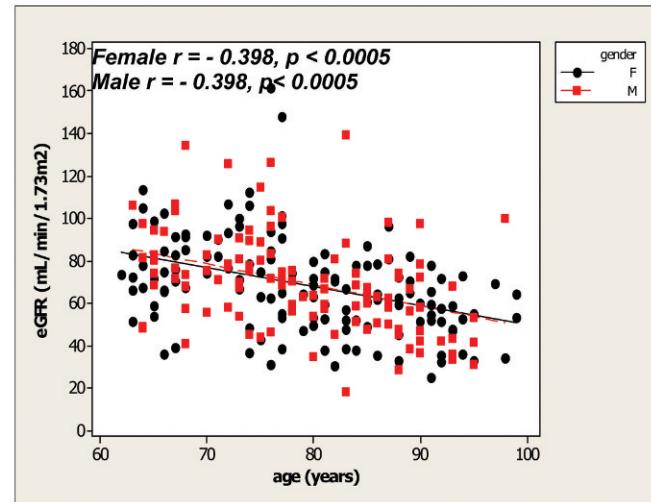
Figure 1 - Aging was associated with a decrease in the estimated glomerular filtration rate (eGFR) in 237 elderly individuals **(A)**. The statistically significant correlation remained when individuals were evaluated according to gender **(B)**. Pearson correlation r = −0.398, p<0.0005.

population (4). Also, it has been reported that patients with chronic kidney diseases present changes in lymphocytes (17). Therefore, in our study population, we investigated whether the confirmed decrease in kidney function (Figures 1A and 1B) was correlated with changes in the levels of CD4+ T, CD8+ T, and B cells.

Figures 2 and 3 show that no significant correlations were observed between eGFR and either the percentage of CD4, CD8, and B cells or CD4/CD8 ratio.

The American Kidney Foundation classification for chronic renal disease according to the estimated glomerular filtration rate is as follows: eGFR between 45 and 60 mL/min/1.73 m^2 = Stage 3A, eGFR between 30 and 45 mL/min/1.73 m^2 = Stage 3B, eGFR between 15 and 30 mL/min/1.73 m^2 = Stage 4 and eGFR<15 mL/min/1.73 m^2 = Stage 5 (end-stage renal disease). Using this classification, we

evaluated the percentages of T and B cells according to eGFR values, where values <60 mL/min/1.73 m^2 and ≥60 mL/min/1.73 m^2 represented chronic kidney disease and the absence of chronic kidney disease, respectively. In our study population, 148 individuals presented with an eGFR≥60 mL/min/1.73 m^2.

In Figure 4, it can be observed that an eGFR≥60 mL/min/1.73 m^2 was associated with a significantly lower median percentage of CD8+ T cells whereas no significant differences were found with respect to the CD4+ T cell percentage, B cell (CD19+) percentage or CD4/CD8 ratio between individuals with similar renal functions. However, we observed a tendency toward higher median percentages of CD4+ and CD19+ cells in individuals with an eGFR≥60 mL/min/1.73 m^2 compared with the percentages in individuals with an eGFR<60 mL/min/1.73 m^2.
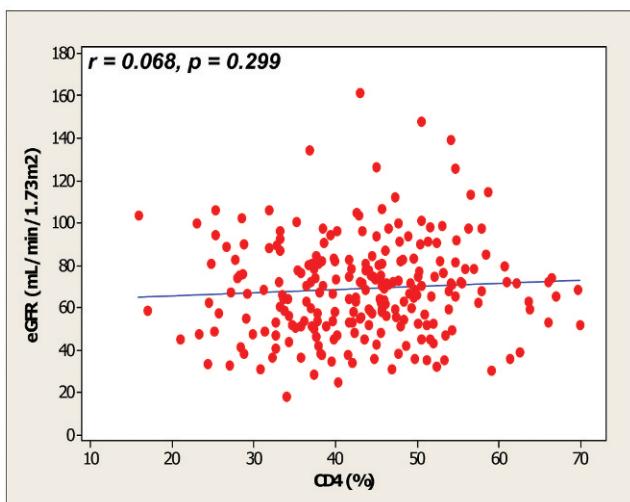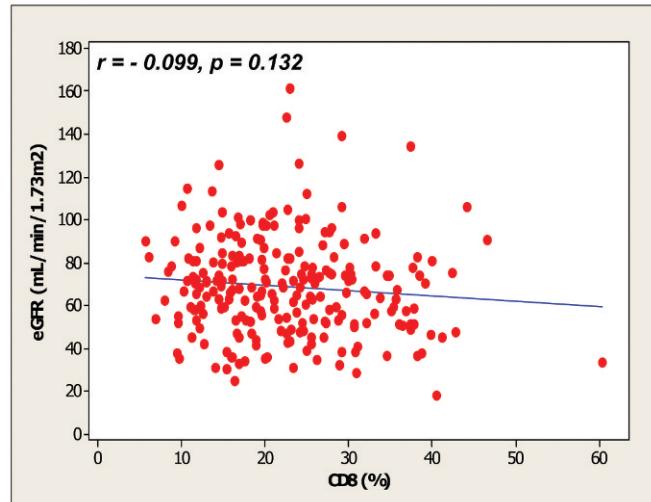
Figure 2 - Estimated glomerular filtration rate (eGFR) *versus* percentage of CD4 T **(A)** and CD8 T **(B)** lymphocytes in 237 elderly individuals. Pearson correlation r = 0.068, p = 0.299 **(A)**; Pearson correlation r = −0.099, p = 0.132 **(B)**.
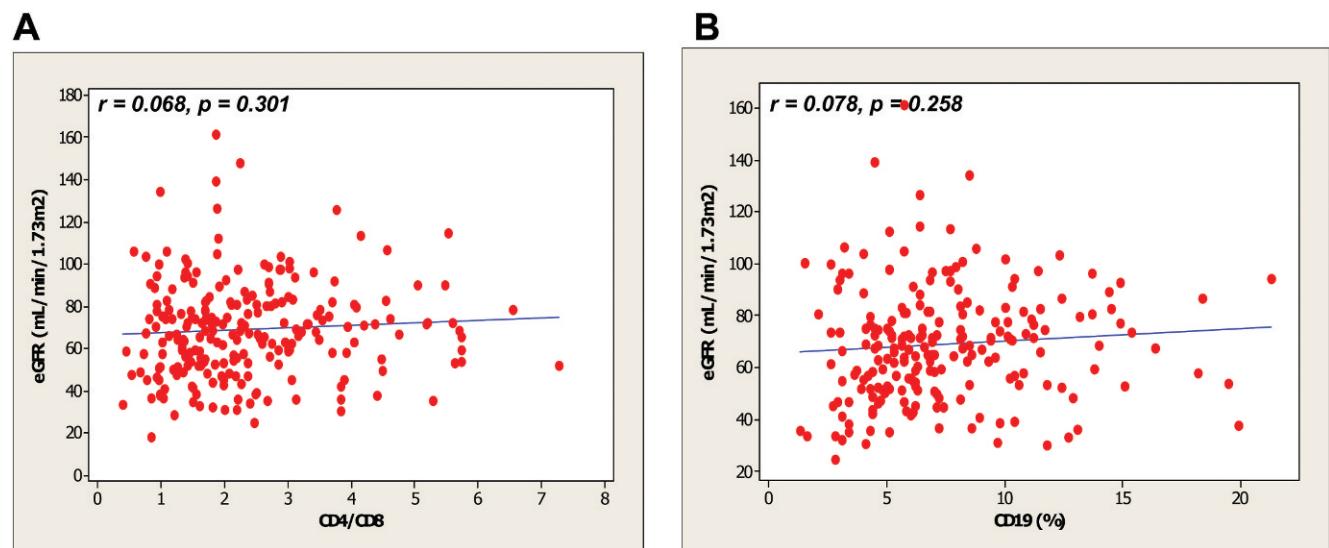
Figure 3 - Estimated glomerular filtration rate (eGFR) *versus* the CD4/CD8 ratio **(A)** and percentage of CD19 B lymphocytes **(B)** in 237 elderly individuals. Pearson correlation r = 0.068, *p* = 0.301 **(A)**; Pearson correlation r = 0.078, *p* = 0.258 **(B)**.
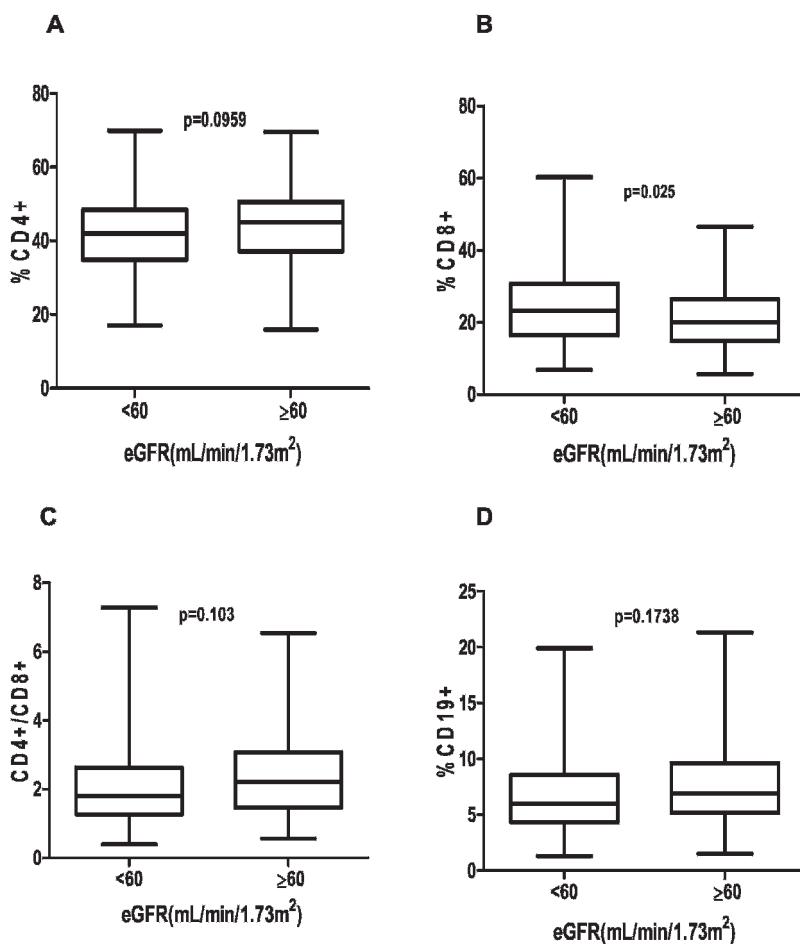
Figure 4 - Median percentage of CD4+, CD8+, and CD19+ cells and the CD4/CD8 ratio in elderly individuals grouped according to whether they had an eGFR<60 mL/min/1.73 m^2 or an eGFR≥60 mL/min/1.73 m^2.

Additionally, the median CD4/CD8 ratio was higher in individuals with an eGFR≥60 mL/min/1.73 m^2.

■ DISCUSSION

Our results are in agreement with those of other studies showing that an increase in age is associated with a decrease in kidney function (1,2).

Patients with chronic kidney disease have been reported to present with immune system changes, such as lower percentages of T and B cells. Chung et al. (18) observed that healthy individuals (creatinine: 0.9 mg/dL) presented with a higher percentage of naïve CD4+ T cells (38.9%) than patients with chronic kidney disease (creatinine: dialysis = 9.7 mg/dL and pre-dialysis = 8.9 mg/dL; CD4+ T cells: dialysis = 31% and pre-dialysis = 25%).

In the present study, no significant correlation was observed between the eGFR calculated using the MDRD equation based on creatinine levels and either CD4+, CD8+, and CD19+ cell percentages or the CD4/CD8 ratio. In elderly individuals, the decline in creatinine clearance and the greater individual variability associated with discrepancies in measured GFR and estimated GFR are factors that contribute to the inconsistency in results. Moreover, most of the individuals studied (n = 148) presented with an eGFR greater than 60 mL/min/1.73 m^2, and, as reported by Van Pottelbergh et al. (19), individuals in this age range with an eGFR≥60 mL/min/1.73 m^2 have low probability of developing chronic renal disease.

When we compared individuals with an eGFR indicating chronic renal disease (>60 mL/min/1.73 m^2) and individuals with an eGFR not suggestive of renal disease (≤60 mL/min/1.73 m^2), a tendency toward higher median levels of CD4+ and CD19+ cells was observed in individuals with better renal function. In addition, the median number of CD8+ cells was significantly higher in individuals with worse renal function.

In contrast to our findings, CD8+ T cell levels have been shown to be decreased in patients with chronic kidney disease (18,20,21), and, consequently, the CD4/CD8 ratio is also decreased (22,23).

Compared with healthy controls, a decrease in the percentage of B cells (CD19+) has been observed in patients with chronic kidney disease (8,16). Considering the important role of B cell-producing antibodies, these findings should be considered in the development of new vaccines. For example, Yaghoubian et al. (23) showed that the mortality of patients 65 years or older admitted for general surgery with a serum creatinine level greater than 2.0 mg/dL was 42% and was associated with infections.

It should be noted that the changes observed in the immune system (immunosenescence) could have several causes, and the reason why some individuals are more protected than others in this process still needs to be clarified. Additionally, it has to be considered that this study has some deficiencies, such as the use of serum creatinine levels in elderly individuals, which could underestimate kidney function even when the results are adjusted for age and gender; the evaluation of immune cells in blood only instead of also investigating them in lymphoid organs (bone marrow, thymus, spleen, and lymph nodes); and the observed changes in the blood phenotype (CD4+, CD8+, and CD19+ cell percentages and the D4/CD8 ratio) that are known to be associated with cell function (proliferation after a stimulus, cytokine production, and apoptosis).

■ AUTHOR CONTRIBUTIONS

Teixeira D and Longo-Maugeri IM performed flow cytometry and contributed to the manuscript writing. Duarte YA and Lebrão ML designed and coordinated the study, collected blood and contributed to the manuscript writing. Bueno V performed flow cytometry, conducted the statistical analysis and contributed to the manuscript writing.

■ REFERENCES

1. Lindeman RD, Tobin J, Shock NW. Longitudinal studies on the rate of decline in renal function with age. J Am Geriatr Soc. 1985;33(4):278-85.
2. Zhang QL, Rothenbacher D. Prevalence of chronic kidney disease in population based studies: systematic review. BMC Public Health. 2008;8:117, http://dx.doi.org/10.1186/1471-2458-8-117.
3. Carson PJ, Nichol KL, O'Brien J, Hilo P, Janoff EN. Immune function and vaccine responses in healthy advanced elderly patients. Arch Intern Med. 2000;160(13):2017-24, http://dx.doi.org/10.1001/archinte.160.13.2017.
4. Teixeira D, Longo-Maugeri IM, Santos JLF, Duarte YAP, Lebrão ML, Bueno V. Evaluation of lymphocyte levels in a random sample of 218 elderly individuals from São Paulo city. Rev Bras Hematol Hemoter. 2011;33(5):367-71, http://dx.doi.org/10.5581/1516-8484.20110100.
5. Vaziri ND, Pahl MV, Crum A, Noris K. Effect of uremia on structure and function of immune system. J Ren Nutr. 2012;22(1):149-56.
6. Sauce D, Larsen M, Fastenackels S, Duperrier A, Keller M, Grubeck-Loebesntein B, et al. Evidence of premature immune aging in patients thymectomized during early childhood. J Clin Invest. 2009;119(10):3070-8, http://dx.doi.org/10.1172/JCI39269.
7. Ogawa T, Kitagawa M, Hirokawa K. Age-related changes of human bone marrow: a histometric estimation of proliferative cells, apoptotic cells, T cells, B cells and macrophages. Mech Ageing Dev. 2000;117(1-3):57-68, http://dx.doi.org/10.1016/S0047-6374(00)00137-8.
8. Guo CH, Chen PC, Yeh MS, Hsiung DY, Wang CL. Cu/Zn ratios are associated with nutritional status, oxidative stress, inflammation, and immune abnormalities in patients on peritoneal dialysis. Clin Biochem 2011;44(4):275-80, http://dx.doi.org/10.1016/j.clinbiochem.2010.12.017.
9. Verkade MA, van de Wetering J, Klepper M. Peripheral blood dendritic cells and GM-CSF as an adjuvant for hepatitis B vaccinations in hemodialysis patients. Kidney Int. 2004;66(2):614-21, http://dx.doi.org/10.1111/j.1523-1755.2004.00781.x.
10. Litiens NH, Huisman M, van der Dorpel M. Impaired immune responses and antigen-specific memory CD4+ T cells in hemodyalysis patients. J Am Soc Nephrol. 2008;19(8):1483-90, http://dx.doi.org/10.1681/ASN.2007090971.
11. Betjes MG, Langerak AW, van der Spek A, de Wit EA, Litjens NH. Premature aging of circulating T cells in patients with end-stage renal disease. Kidney Int. 2011;80(2):208-17, http://dx.doi.org/10.1038/ki.2011.110.
12. Libetta C, Rampino T, Dal Canton A. Polarization of T-helper lymphocytes toward the Th2 phenotype in uremic patients. Am J Kidney Dis. 2001;38(2):286-95.
13. Milne CD, Paige CJ. IL-7: a key regulator of B lymphopoiesis. Semin Immunol. 2006;18(1):20-30, http://dx.doi.org/10.1016/j.smim.2005.10.003.
14. Kalled L. Impact of the BAFF/BR3 axis on B cell survival, germinal center maintenance and antibody production. Semin Immunol. 2006;18(5):290-6, http://dx.doi.org/10.1016/j.smim.2006.06.002.
15. Bouts A, Davin J, Krediet R. Children with chronic renal failure have reduced numbers of memory B cells. Clin Exp Immunol. 2004;137(3):589-94, http://dx.doi.org/10.1111/j.1365-2249.2004.02571.x.
16. Pahl MV, Gollapudi S, Sepassi L, Gollapudi P, Elahimehr R, Vaziri ND. Effect of end-stage renal disease on B-lymphocyte subpopulations, IL-7, BAFF and BAFF receptor expression. Nephrol Dial Transplant 2010;25(1):205-12, http://dx.doi.org/10.1093/ndt/gfp397.
17. Chung BH, Kim KW, Sun IO, Choi SR, Park HS, Jeon EJ, et al. Increased interleukin-17 producing effector memory T cells in the end-stage renal disease patients. Immunol Lett. 2012;141(2):181-9, http://dx.doi.org/10.1016/j.imlet.2011.10.002.
18. Van Pottelbergh G, Bartholomeeeusen S, Buntix F, Degryse J. The evolution of renal function and the incidence of end-stage renal disease

in patients aged ≥50 years. Nephrol Dial Transplant. 2011;27(6):2297-303.

19. Litjens NH, de Wit EA, Betjes MG. Differential effects of age, cytomegalovirus-seropositivity and end-stage renal disease (ESRD) on circulating T lymphocyte subsets. Immun Ageing. 2011;8(1):2, http://dx.doi.org/10.1186/1742-4933-8-2.

20. Vacher-Coponat H, Brunet C, Lyonnet L, Bonnet E, Loundou A, Sampol J, et al. Natural killer cell alterations correlate with loss of renal function and dialysis duration in uraemic patients. Nephrol Dial Transplant. 2008;23(4):1406-14.

21. Saurwein-Teissl M, Lung TL, Marx F, Gschosser C, Asch E, Blasko I. Lack of antibody production following immunization in old age: association with CD8(+) CD28(-) T cell clonal expansions and an imbalance in the production of Th1 and Th2 cytokines. J Immunol 2002;168(11):5893-9.

22. Yoon J-W, Gollapudi S, Pahl MV, Vaziri ND. Naïve and central memory T-cell lymphopenia in end-stage renal disease. Kidney Int. 2006;70(2):371-6, http://dx.doi.org/10.1038/sj.ki.5001550.

23. Yaghoubian A, Ge P, Tolan A, Saltmarsh G, Kaji AH, Neville AL, et al. Renal insufficiency predicts mortality in geriatric patients undergoing emergent general surgery. Am Surg. 2011;767(10):1322-5.