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The Study of Relationship between Serum Levels of Soluble fms-like Tyrosine Kinase-1 and Soluble Fibrinogen-like Protein 2 with Delayed Graft Function after Kidney Transplantation

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ABSTRACT

Delayed graft function (DGF) is a transplant complication which means a need to dialysis throughout the first week after transplantation. This study aimed to ascertain the relationship between the two immunomodulatory factors of soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble fibrinogen-like protein 2 (sFGL-2) with DGF after transplantation.

This case-control study was done in 2 groups of 58 kidney transplant patients with and without DGF. The control group included the patients who didn't show DGF symptoms. Then, serum levels of sFlt-1and sFGL-2 in all blood samples were measured by ELISA.

Serum sFlt-1 and sFGL-2 levels were significantly higher in the DGF group compared to those in the control group ($p \leq 0.001$). sFlt-1 and sFGL-2 serum levels significantly affect DGF ($p < 0.001$) in such a way that they may be diagnostic factors of DGF.

This study showed a significant relationship between sFlt-1 as well as sFGL-2 and DGF. Therefore, plasma levels of sFlt-1 and sFGL-2 may be used as diagnostic tools to determine the risk of DGF.

Keywords: Delayed graft function; Kidney transplantation; Soluble VEGF receptor-1; Soluble fibrinogen-like protein 2

INTRODUCTION

Human kidney transplantation is the most effective treatment of chronic advanced renal failure. Although this method relieves patients of many complications of dialysis, it has some complications on its own, one of which is Delayed Graft Function (DGF)[1] accelerating 10% rate of rejection. While the traditional definition of DGF rests on dialysis requirement during the first postoperative week, it has been functionally defined as increase of serum creatinine at least 10% daily on 3 successive days during the first week post-transplantation.[2] Recent studies have proved some relationships between DGF and long term complications of transplantation[3]

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including decreased graft survival and increased risk of graft rejection.[4]

Vascular endothelial growth factor (VEGF) is an important growth factor which is secreted by many cells and takes part in angiogenesis.[5] There are 3 main VEGF receptor subgroups which may be membrane-bound or soluble.[6] Soluble fms-like tyrosine kinase-1 (sFlt-1) is a splice variant of VEGF receptor-1[7] which is produced by endothelial cells, monocytes and platelets and, as a potent antagonist of VEGF, binds and sequesters VEGF. Thus, the effectiveness of VEGF would be reduced in the presence of increased levels of sFlt-1. Considering the inflammatory effects of VEGF,[8] we may assume that sFtl-1 modulates the immune responses in some pathologies.[9,10]

Soluble Fibrinogen-Like protein 2 (sFGL-2), as a soluble member of the superfamily of proteins related to fibrinogen, is a molecule holding regulatory capabilities in the immune system and thereby is mainly secreted by regulatory T (Treg) cells including $CD4^{+}CD25^{+}FoxP3^{+}$ and $CD8^{+}CD45RO^{low}$ ones.[11,12] In fact, this factor induces the apoptosis of different immune cells[13-16] and in this way, it protects the tissue to be damaged[17] in different pathologies such as hepatitis C[18] and transplantation.[19,20] Although the signaling process of sFGL-2 is still unclear, its relation to Mitogen-activated Protein Kinase (MAPK) signaling pathway[21] induces the inhibitory functions of Th17/Treg cells in different pathologies such as tumors.[22]

sFlt-1 and sFGL-2 have not been addressed as effective factors on the pathologic condition of DGF so far. Changed levels of sFlt-1and sFGL-2 in DGF patients compared to those in non-DGF patients may endorse such effectiveness. If so, modulators of such factors may be able to affect DGF fate; and in this way, they may accelerate the recovery of kidney function to normal state after transplantation.

MATERIALS AND METHODS

Study Population

This retrospective study was performed on 2 groups of 58 first kidney-transplant adults with and without DGF at Baqiyatallah hospital, Tehran, during 2016. Both groups had a living donor and were matched according to age, sex, family relation to the donor, panel reactive antibody (PRA), no history of transfusion, and the rate of HLA mismatch. Having excluded any patient with hyperkalemia, urinary as well as cardiovascular disease, and any cause of renal dysfunction including surgical complications and renal vein or artery thrombosis, we confirmed the diagnosis of DGF according to each way of a) need for dialysis in the first week after transplantation, b) increasing more than or decreasing less than 10% of serum creatinine within 3 days' post-transplant. The control group was selected from the patients who underwent a transplant and did not show the mentioned DGF criteria at the end of the first week. The induction, as well as maintenance immunosuppression protocol for all recipients, was composed of therapeutic adjusted doses of calcineurin inhibitors, mycophenolate mofetil, and steroids along with the study. There was no event of cytomegalovirus (CMV) and BK infection according to standard criteria, urinary and respiratory infections, and biopsy-proven acute rejection in both DGF and control groups. The study protocol was confirmed by the local ethics committee (N. KAUMS:3537). Written informed consent was obtained from all participants.

Measurements

A serum sample was prepared from all 116 participants as soon as DGF was diagnosed in the DGF group and at the end of the first week in the control group. Samples were kept at -20 until analysis. Once collected, the samples were sent to laboratory and serum levels of sFlt-1 and sFGL-2 were evaluated by sandwich ELISA, eBioscience USA, according to kit recommendations.

Statistics

The results were expressed as mean±SD. Kolmogorov Simonov test for Normality of the quantitative variables was assessed. The groups were compared by independent *t*-test and differences in proportions were tested using Chi-square and Fisher's exact test. Multiple binary logistic regression analysis was used to adjust the effect of probable confounding variables. Using ROC and Area under Curve, we tried to determine the sensitivity and specificity of sFlt-1 and sFGL-2 in the diagnosis of DGF. $p<0.05$ was considered statistically significant. All analyses were made by the SPSS package (version 16; Statistical Package for the Social Sciences, SPSS Inc., Chicago, Illinois, USA).

RESULTS

Basic and Clinical Characteristics of the Study Groups

Basic and clinical characteristics of the study groups are shown in Table 1. Serum sFlt-1 and sFGL-2 levels were significantly higher in the DGF group compared to those in the control group (p<0.001).

Serum levels of sFlt-1 and sFGL-2 were significantly higher in the DGF group compared to control ones according to different demographic and clinical parameters (p<0.01) (Table 2).

Evaluating the Effect of Different Variables on DGF

Using multiple logistic regression model to evaluate the effect of different confounding variables of age, gender, family relationship between donor and recipient, HLA-mismatch, percentage of PRA, type of medication, sFlt-1 and sFGL-2 on the presence or absence of DGF (as the dependent variable), we showed that DGF is affected by sFlt-1 (p=0.003) and sFGL-2 (p=0.002) levels. The model illustrated that an increase in one year of the recipient's age increases the DGF risk as much as 17% (OR=1.17), and one-unit increase of GFR decreases the risk of DGF as low as 0.944 times. The DGF risk also decreases with cyclosporine consumption (OR=0.091). It was found that each unit of increase in sFGL-2 and sFlt-1 levels significantly increases the risk of DGF 1.10 times (p=0.002) and 1.045 times (p=0.003), respectively. No significant effects of the other factors were found. However, our model showed a high power of expression (Nagelkerke R Square=0.872) and proper goodness of fit (p<0.001). GFR had a significant effect on DGF (p=0.015) in a way that any unit increase in GFR increased the risk of DGF by 6% (Table 3).

Cut-off Points and Predictive Values

Using ROC curve and Area under Curve (AUC), we determined both sensitivity and specificity of sFlt-1 and sFGL-2 in the diagnosis of DGF. Analysis of ROC in cut-off point of 64.7 pg/mL for sFlt-1 showed 91.4% sensitivity and 74.1% specificity (AUC=0.955). Positive predictive value (PPV) and negative predictive value (NPV) of the test were 90.5% and 98.1%, respectively (Figure 1).

Analysis of ROC showed 98.3% sensitivity and 89.7% specificity at the cut-off point of 55.9 for sFGL-2 in the diagnosis of DGF (AUC=0.966) (Figure 1). Positive predictive value (PPV) and negative predictive value (NPV) of the test were 90.5% and 98.1%, respectively.

Table 1. Basic and clinical characteristics of the patients with or without delayed graft function (DGF)

Scale	Variable	DGF	Without DGF	*p* value
Demographic	Age (Mean±SD)	45.5±11.6	45.6±11.7	0.949
characteristics	Sex (Male%)	37(63.8%)	27(46.6%)	0.062
	Family relation	44(75.9)	50(86.2)	0.155
	HLA mismatch (Mean±SD)	3.83±0.92	3.53±0.98	0.099
	GFR	48.92±16.13	50.73±18.46	0.575
Biochemistry	LDL mg/dl (Mean±SD)	131.1±48.6	122.3±45.1	0.317
characteristics	ALT IU/L(Mean±SD)	41.8±22.9	40.7±27.9	0.805
	AST IU/L(Mean±SD)	31.1±14.1	32.6±14.3	0.058
	K^+ mEq/L (Mean±SD)	4.4±0.42	4.3±0.49	0.394
	Cr mg/dl (Mean±SD)	1.88±1.36	1.66±1.22	0.345
Cell counts	WBC (Mean±SD)	7.38±2.63	8.45±3.5	0.064
	Platelets (Mean±SD)	18.1±6.2	19.6±6.4	0.218
	Hb g/dl (Mean±SD)	12.2±2.39	11.7±1.7	0.192
sFlt-1pg/mL (Mean±SD)		142.3±52.5	53.1±17.4	<0.001
sFGL-2 pg/mL (Mean±SD)		80.34 ± 20.25	40.44 ± 14.73	<0.001

DGF: Delayed Graft Function, HLA: Human Leukocyte Antigen, GFR: Glomerular Filtration Rate, LDL: Low Density Lipoprotein, ALT: Alanine Amino Transferase, AST: Aspartate amino Transferase, K^+: Potassium, Cr: Creatinine, WBC: White Blood Cells, Hb: Hemoglobin, sFlt-1: soluble fms-like tyrosine kinase-1, sFGL-2: soluble Fibrinogen-like protein 2

Table 2. Serum levels of sFlt-1 and sFGl-2 according to different variables in patients with or without delayed graft function (DGF)

Variable			With DGF (Mean±SD)	Without DGF (Mean±SD)	*p* value
Age (year)	<40	sFlt-1	141.9±48.7	54.3±16.4	<0.001
		sFGL-2	79.10 ± 20.33	43.29 ± 15.44	<0.001
	≥40	sFlt-1	142.4±55.1	52.2±18.2	<0.001
		sFGL-2	85.58 ± 20.12	38.71 ± 14.23	<0.001
Sex	Male	sFlt-1	150.4±53.2	51.4±17.3	<0.001
		sFGL-2	84.11 ± 19.47	39.99 ± 14.87	<0.001
	Female	sFlt-1	127.8±49.4	54.5±17.6	<0.001
		sFGL-2	81.99 ± 21.99	40.84 ± 14.85	<0.001
HLA mismatch	<3 mismatches	sFlt-1	133.6±43.9	56.4±16.1	<0.001
		sFGL-2	77.96 ± 22.04	49.19 ± 16.35	0.009
	≥3 mismatches	sFlt-1	145.2±55.40	50.3±18.2	<0.001
		sFGL-2	84.08 ± 20.12	38.84 ± 14.01	<0.001
Family relation	Yes	sFlt-1	134.6±53.2	53.5±18.3	<0.001
		sFGL-2	79.89 ±17.51	39.79 ± 15.04	<0.001
	No	sFlt-1	166.4±43.9	50.1±10.8	<0.001
		sFGL-2	94.18 ± 24.85	44.53 ± 12.72	<0.001
Drugs used	Cyclosporine	sFlt-1	155.3±49.4	55.3±17.6	<0.001
		sFGL-2	87.81 ± 21.71	40.62 ± 13.95	<0.001
	Tacrolimus	sFlt-1	76.6±28.4	44.5±18.8	0.008
		sFGL-2	79.34 ± 20.44	39.86 ± 20.21	0.001

HLA: Human Leukocyte Antigen, sFlt-1: soluble fms-like tyrosine kinase-1, sFGL-2: soluble Fibrinogen-like protein 2

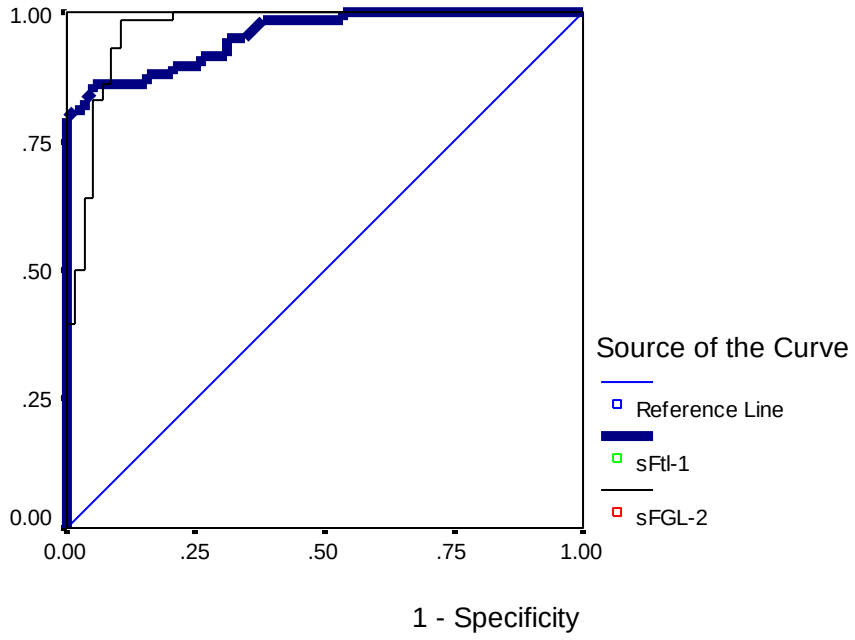

Figure 1. Receiver operating characteristic (ROC) of sFtl-1 and sFGL-2 for diagnosis of delayed graft function (DGF)

Table 3. Multiple logistic regression model for evaluating the effect of different variables on delayed graft function (DGF)

Independent variables	Coefficients(B)	S.E. of B	Sig.	Exp(B)
Age of recipient	0.156	0.071	0.028	1.169
Cyclosporine consumption	-2.399	0.826	0.004	0.091
GFR	0.057	0.024	0.015	1.059
sFGL-2	0.100	0.032	0.002	1.105
sFlt-1	0.044	0.015	0.003	1.045

GFR: Glomerular Filtration Rate, sFlt-1: soluble fms-like tyrosine kinase-1, sFGL-2: soluble Fibrinogen-like protein 2

DISCUSSION

Our study demonstrated that sFlt-1serum levels are associated with DGF after kidney transplant. This result is in line with other studies. For example, in a relevant study, patients with DGF showed higher plasma levels of sFlt-1.[23] sFlt-1 (as a VEGF receptor) traps VEGF which is an angiogenic factor facilitating immune cell migration and adhesion; therefore, sFlt-1 may negatively regulate the inflammatory condition of graft dysfunction in organs like lungs[24] and kidneys experiencing DGF. Such result is confirmed by the observation that a recombinant version of natural sFlt-1[25,26] as well as its adenovirus- or plasmid-mediated gene transfer[27] may inhibit angiogenesis and inflammatory indices. Similarly, antagonizing VEGFR-2 by a soluble VEGFR-2 recombinant chimeric protein ameliorates graft rejection in mice undergoing corneal transplant[28] which is well in line with a predominant anti-inflammatory mode of action of this treatment. Showing a significant positive correlation between sFlt-1 and DGF in our patients, we may consider that following DGF, sFlt-1 increases likely as a protective mechanism ameliorating the disease pathology. This might explain the reason that murine collagen-induced arthritis could be therapeutically inverted via sFlt-1.[29]

This study was also designed to examine, for the first time, the possible effect of sFGL-2 on DGF. sFGL-2 is secreted from Tregs which have regulatory capabilities in some pathologic conditions. For example, previous studies have shown significantly increased serum level of sFGL-2 in allograft kidney transplant recipients with acute rejection[30,31] perhaps due to the fact that the host tries to compensate for the injury during acute rejection process.[32,33] Showing a significant positive correlation between sFGL-2 and DGF in our patients, we may consider that following DGF, sFGL-2 increases likely as a protective mechanism ameliorating the disease pathology. Actually, the emergence of more sFGL-2 in splenic Tregs of transplant models indicates the involvement of such factor in inhibitory functions of Tregs[34] as well as long-term survival after transplantation.[35] Although the sFGL-2 impact is uncertain on B cells, it may protect the graft through apoptosis of such cells.[36]

Our study suffered from some limitations. First, functional assays which provide further information on the immunomodulatory effect of sFlt-1, as well as sFGL-2 on DGF, were not performed. Second, we missed an extra checkpoint sampling. Further control points yield a better assessment of such factors in the prediction of DGF.

The results of our study showed an increased serum level of sFlt-1 as well as sFGL-2 associated with DGF. Considering their inhibitory roles, we may consider a protective role for both molecules among DGF. This concept may confirm the beneficial effects of sFlt-1 and sFGL-2 as diagnostic factors as well as novel therapeutics in DGF conditions after transplantation.

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REFERENCES

1. Koning OH, Ploeg RJ, van Bockel JH, Groenewegen M, et al. Risk factors for delayed graft function in cadaveric kidney transplantation: a prospective study of renal function and graft survival after preservation with University of Wisconsin solution in multi-organ donors.Transplantation 1997; 63(11):1620-8.
2. Moore J, shabir S, Chand S, Bentall A, McClean A, Chan W, et al. Assessing and comparing rival definitions of delayed renal allograft function for predicting subsequent graft failure. Transplantation 2010; 90(10):1113-6.

3. Holmes K, Roberts OL, Thomas AM, Cross MJ .Vascular endothelial growth factor receptor-2: structure, function, intracellular signaling and therapeutic inhibition. Cell Signal 2007; 19(10):2003-12.
4. Danovitch G. Handbook of kidney transplantation. 4th ed. Philadelphia, LipimcottWilliams &Wilkins; 2005.
5. Ribatti D, Tamma R. Hematopoietic growth factors and tumor angiogenesis. Cancer Lett 2018; S0304-3835(18):30614-1.
6. Tuder RM, Flook BE, Voelkel NF. Increased gene expression for VEGF and the VEGF receptors KDR/flk and flt in lungs exposed to acute or chronic hypoxia. J Clin Invest 1995; 95(4):1798-807.
7. Roberts WG, Palade GE. Increased microvascular permeability and endothelial fenestration induced by vascular endothelial growth factor. J cell Sci 1995; 108(6):2369-79
8. Schweighofer B, Testori J, Sturtzel C, Sattler S, Mayer H, Wagner O, et al. The VEGF-induced transcriptional response comprises gene clusters at the crossroad of angiogenesis and inflammation. Thromb Haemost 2009; 102(3):544-54.
9. Ataga KI, Brittain JE, Jones SK, May R, Delaney J, Strayhorn D,et al. Association of soluble fms-like tyrosine kinase-1 with pulmonary hypertension and haemolysis in sickle cell disease. Br J Haematol 2011; 152(4):485-91.
10. Kulkarni1 A, Mehendale S, YadavH, KilariA, Taralekar V, Joshi S. Circulating angiogenic factors and their association with birth outcomes in preeclampsia. Hypertens Res 2010; 33(6):561-7.
11. Shalev I, Liu H, Koscik C, Bartczak A, Javadi M, Wong KM, et al. Targeted deletion of fgl2 leads to impaired regulatory T cell activity and development of autoimmune glomerulonephritis. J Immunol 2008; 180(1):249-60.
12. Li XL, Ménoret S, Bezie S, Caron L, Chabannes D, Hill M, et al. Mechanism and localization of CD8 regulatory T cells in a heart transplant model of tolerance. J Immunol 2010; 185(2):823-33.
13. Wang L, Yang C, Xu M, Hu M, Wang X, Zhu T. The Role of Soluble Fibrinogen-Like Protein 2 in Transplantation: Protection or Damage. Transplantation 2014; 97(12):1201-6.
14. Selzner N, Liu H, Boehnert MU, Adeyi OA, Shalev I, Bartczak AM, et al. FGL2/fibroleukin mediates hepatic reperfusion injury by induction of sinusoidal endothelial cell and hepatocyte apoptosis in mice. J Hepatol 2012; 56(1):153-9.
15. Radeke HH, Janssen-Graalfs I, Sowa EN, et al. Opposite regulation of type II and III receptors for immunoglobulin G in mouse glomerular mesangial cells and in the induction of anti-glomerular basement membrane (GBM) nephritis. J Biol Chem 2002; 277(30):27535-44.
16. Liu H, Zhang L, Cybulsky M, Gorczynski R, Crookshank J, Manuel J, et al. Identification of the receptor for FGL2 and implications for susceptibility to mouse hepatitis virus (MHV-3)-induced fulminant hepatitis. Adv Exp Med Biol 2006; 581:421-5.
17. Zhao Z, Wang L, Yang C, Zhao T, Li L, Hu L, et al. Soluble FGL2 induced by tumor necrosis factor-α and interferon-γ in CD4+ T cells through MAPK pathway in human renal allograft acute rejection. J Surg Res 2013; 184(2):1114-22.
18. Foerster K, Helmy A, Zhu Y, Khattar R, Adeyi OA, Wong KM, Shalev I, Clark DA, Wong PY, Heathcote EJ, Phillips MJ, Grant DR, Renner EL, Levy GA, Selzner N. The novel immunoregulatory molecule FGL2: a potential biomarker for severity of chronic hepatitis C virus infection. J Hepatol 2010; 53(4):608–15.
19. Xie L, Ichimaru N, Morita M, Chen J, Zhu P, Wang J, et al. Identification of a novel biomarker gene set with sensitivity and specificity for distinguishing between allograft rejection and tolerance. Liver Transpl 2012; 18(4):444–5429.
20. Zhao Z, Yang C, Tang Q, Zhao T, Jia Y, Ma Z, et al. Serum level of soluble fibrinogen-like protein 2 in renal allograft recipients with acute rejection: a preliminary study. Transplant Proc 2012; 44(10):2982-5.
21. Liu H, Shalev I, Manuel J, He W, Leung E, Crookshank J, et al. The FGL2- FcγRIIB pathway: A novel mechanism leading to immunosuppression. European journal of immunology 2008; 38(11):3114-26.
22. Zhang N, Schröppel B, Lal G, Jakubzick C, Mao X, Chen D, et al. Regulatory T cells sequentially migrate from inflamed tissues to draining lymph nodes to suppress the alloimmune response. Immunity 2009;30(3):458-69.
23. Chapal M, Néel M, Le Borgne F, Meffray E, Carceles O, Hourmant M, et al. Increased Soluble Flt-1 Correlates With Delayed Graft Function and Early Loss of Peritubular Capillaries in the Kidney Graft. Transplantation 2013; 96(8):739-44.
24. Krenn K1, Klepetko W, Taghavi S, Lang G, Schneider B, Aharinejad S,et al. Recipient vascular endothelial growth factor serum levels predict primary lung graft dysfunction.Transplantation 2007; 7(3):700-6.
25. Takayama K, Ueno H, Nakanishi Y, Sakamoto T, Inoue K, Shimizu K, et al. Suppression of tumor angiogenesis

and growth by gene transfer of a soluble form of vascular endothelial growth factor receptor into a remote organ. Cancer Res 2000; 60(8):2169-77.
26. Honda M, Sakamoto T, Ishibashi T, Inomata H, Ueno H. Experimental subretinal neovascularization is inhibited by adenovirus-mediated soluble VEGF/flt-1 receptor gene transfection: a role of VEGF and possible treatment for SRN in age-related macular degeneration. Gene Ther 2000; 7(11):978-85.
27. Zhu C-S, Hu X-Q, Xiong Z-J, Lu Z-Q, Zhou G-Y, Wang D-J. Adenoviral delivery of soluble VEGF receptor 1 (sFlt-1) inhibits experimental autoimmune encephalomyelitis in dark Agouti (DA) rats. Life Sci 2008; 83(11):404-12.
28. Hayashi T1, Usui T, Yamagami S.Suppression of Allograft Rejection with Soluble VEGF Receptor 2 Chimeric Protein in a Mouse Model of Corneal Transplantation.Tohoku J Exp Med 2016; 239(1):81-8.
29. Miotla J, Maciewicz R, Kendrew J, Feldmann M, Paleolog E. Treatment with soluble VEGF receptor reduces disease severity in murine collagen-induced arthritis. Lab Invest 2000; 80(8):1195-205.
30. Zhao Z, Yang C, Tang Q, Zhao T, Jia Y, Ma Z, et al. Serum level of soluble fibrinogen-like protein 2 in renal allograft recipients with acute rejection: a preliminary study. Transplant Proc 2012; 44(10):2982-5.
31. Ning Q, Sun Y, Han M, et al. Role of fibrinogen-like protein 2 prothrombinase/fibroleukin in experimental and human allograft rejection. J Immunol 2005; 174(11):7403-11.
32. Aquino-Dias EC, Joelsons G, da Silva DM, Berdichevski RH, Ribeiro AR, Veronese FJ, Goncalves LF, Manfro RC. Non-invasive diagnosis of acute rejection in kidney transplants with delayed graft function. Kidney Int 2008; 73(7):877–84
33. Taflin C, Nochy D, Hill G, Frouget T, Rioux N, Verine J, Bruneval P, Glotz D. Regulatory T cells in kidney allograft infiltrates correlate with initial inflammation and graft function. Transplantation 2010; 89(2):194–9.
34. Wei S, Kryczek I, Zou W. Regulatory T-cell compartmentalization and trafficking. Blood 2006; 108(2):426-31.
35. Zhang N, Schröppel B, Lal G, Jakubzick C, Mao X, Chen D, et al. Regulatory T cells sequentially migrate from inflamed tissues to draining lymph nodes to suppress the alloimmune response. Immunity 2009; 30(3):458-69.
36. Lieberthal W, Koh JS, Levine JS. Necrosis and apoptosis in acute renal failure. Semin Nephrol 1998; 18(5):505-18.