THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

CLINICAL AND IMMUNOLOGICAL CHARACTERISTICS OF LUPUS NEPHRITIS

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Abreviations

ACR: American College of Rheumatology
AKL: anticardiolipin antibody
C3-, C4: complement
CRP: C-reactive protein
CD: cluster of differentiation
DNS: dezoxiribonucleicacid
GFR: glomerular filtration rate
IC: immuncomplex
ICAM: intercellular adhesion molecule
IFN: interferon
IgG: immunglobulin G
IL: interleukin
IP-10: interferon gamma-inducible protein
ISN/RPS: International Society of Nephrology/Renal Pathology Society
LN: lupus nephritis
MCP-1: monocyte chemotactic protein
MIF: migration inhibitor factor
NIH: National Institute of Health
NGAL: neutrophil-gelatinase-associated lipocalin
SDR: sedimentation rate
SLE: systemic lupus erythematosus
SLEDAI: systemic lupus erythematosus activity index
TNF: tumor necrosis factor
TWEAK: tumor necrosis factor-like inducer of apoptosis
VCAM: vascular adhesion molecule

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic, relapsing, polysystemic autoimmune disease with various clinical signs that affects young women of childbearing age. Initial signs of the disease are not specific: fever, weight loss, fatigue. In most of the patients the first sign is photosensitivity, classic and specific skin abnormality is butterfly erythema. Polyarthritis, serositis is also common.

The prognosis of SLE patients is influenced by neuropsychiatric and renal involvement. Lupus nephritis (LN) is present in 40-60% of patients, 10-15% of patients are developing end stage renal disease. Microscopic hematuria and proteinuria are the most common signs, sometime proteinuria is nephritic, more than 3.5 g/day. Renal biopsy is needed in case of severe persistent proteinuria or deterioration of renal functions. We could distinguish six histological types of lupus nephritis based on International Society of Nephrology/Renal Pathology Society (ISN/RPS): I. minimal mesangial LN, II. mesangial LN, III. focal LN, IV. diffuse proliferative LN, V. membranosus LN, VI. glomerulosclerosis.

Pathogenesis of LN is related to nephritogenic autoantibodies. Autoantibodies against double stranded deoxyribonucleic acid (dsDNA) cross-react with glomerular basement membrane antigens (laminin, phospholipids), antibodies with higher affinity form immuncomplexes intravascularly which deposits in glomeruli, IgG activates the complement system. Different chemokines as monocyte chemoattractant protein (MCP-1), complement 5 factor (C5a), macrophage migration inhibitory factor (MIF) are expressed. The lupus nephritic kidney secretes mainly T-helper 1 type cytokines, in diffuse proliferative LN cells secretes interferon-gamma (IFN-γ) and express CD40. The CD40-CD40L interaction increases IL-12 expression, which induces Th-1 response. Expression of IL-1β is increased mainly in diffuse proliferative form, level of IL-10 is high in SLE leading to an enhanced B-cell activation.

Optimal care of patients with LN is necessary, serum urea, creatinine, glomerular filtration rate (GFR), protein measurement in 24 hour collected urine, urine sediment should be checked regularly. Beside urinary parameters other activity markers should be tested. Anti DNA antibody, complement components, sedimentation rate (SDR), C-reactive protein (CRP), antiphospholipide antibodies, anti-nucleosome antibodies and anti-C1q antibodies correlate to renal activity.

Classical laboratory parameters are not sensitive and specific in prediction renal flares and do not distinguish between activity and chronicity. Chronic proteinuria is not necessary the sign of activity, and the kidney inflammation could be active without worsening of

proteinuria or renal function. Renal histology reflects activity or chronicity, but the renal biopsy is an invasive approach and serial biopsies are not applicable in clinical situations because of side effects.

Over the last few years there has been a growing interest in searching novel lupus biomarkers, which could predict future renal flares.

Urinary proteome techniques are use to distinguish proteins in urine. Concentration of hepcidin 20 increases several months before relapse. Neutrophil gelatinase-associated lipocalin (NGAL), is a small glycosilated protein, concentration of this protein in urine was high in lupus nephritis and correlated with the renal activity.

Cytokines and chemokines are also candidate biomarkers. Urinary concentration of MCP-1 (monocyte chemotactic factors) is high mainly in proliferative forms (LN III and IV), level of TWEAK (tumor necrosis factor-like inducer of apoptosis) correlated with the renal activity, specific to glomerular involvement. Role of IL-1 is well known in tissue damage, the level of soluble IL-2 receptor is high in major relapses, reflects activity of kidneys. IL-6 and IL-10 levels are also higher in lupus nephritis, but there was no significant difference between patients with LN and without. Other biomarkers correlating with kidney activity: IP-10 (interferon gamma inducible protein), serum and urine IL-12, urine VCAM-1 (vascular adhesion molecule), P-selectin, serum intercellular adhesion molecule (ICAM-1), urine endothelin-1, osteoprotegerin.

OBJECTIVES

1. My goal was to find out the renal involvement of a large cohort of SLE patients treated by our department, to analyze the occurrence of histological types, the clinical and immunological characteristics, therapeutical regimens.
2. To find out the correlation between the activity of LN and classical laboratory parameters as serum complement levels, antiDNA antibody level, CRP-level.
3. To compare serum and urinary cytokine levels of patients with and without renal involvement, to find out the best marker which could help the early diagnosis and could help in prediction of renal flares.
4. IL-1Ra level, as a new biomarker was determined in sera of patients with and without LN, to find out whether this cytokine could help in early diagnosis of LN.

PATIENTS AND METHODS

Patients, included in this study fulfilled four or more criteria of the American College of Rheumatology for the classification of SLE [9], diagnosed, treated and followed-up by the 3rd Department of Internal Medicine, Division of Clinical Immunology, University of Debrecen, Hungary between 1974-2005. We analyzed the medical records/charts and digital databases retrospectively using Medsolution medical program and recorded the following: age, gender, time of the diagnosis of SLE and LN, duration of follow-up and therapy. Up to the end of 2004 I analyzed the medical records of 551 SLE patients. The criteria for the diagnosis of LN included persistent proteinuria or proteinuria greater than 0.5 g/day, presence of cellular casts and haematuria, or the patient had signs of nephrotic syndrome. Renal biopsies were performed at the first occurrence of significant proteinuria or in cases of persistent proteinuria if no contraindications presented. The histological findings were recorded based on the classification of the International Society of Nephrology and the Renal Pathology Society.

Laboratory methods

I focused especially on C3 complement level, double-stranded DNA (dsDNA) and anticardiolipin antibodies (AKL) as classical activity markers of the disease. The immunoserological tests were made by Regional Immunological Laboratory. Complement 3 (C3) levels in the sera of patients were measured according to the manufacturer's instructions by nephelometry (normal range: 0.8-1.9 g/l), anti-dsDNA (positive <50 UI/ml) and anti-cardiolipin, anti-beta2-gylcoprotein antibody (both positive if <10 IU/ml) levels were determined by ELISA according to the manufacturer's instructions using internationally approved kits.

We measured the serum and urinary concentration of different cytokines as new biomarkers, using ELISA (Th1-cytokine: IL-2, IFN-γ, TNF-α, Th2-cytokine: IL-4, IL-10, IL-13, moreover IL-1, IL-6, IL-8 and TGF-β). Interleukin1-receptor antagonist (IL-1Ra) level was determined by Fluorokine MAP cytokine multiplex kits designed for Luminex 100^{TM} analyzer using analyte-specific antibodies.

Routine laboratory tests as serum creatinine, glomerular filtration rate (GFR), CRP levels were measured by Clinical Biochemical and Molecular Pathology Department.

Disease activity was calculated using systemic lupus erythematosus disease activity index (SLEDAI).

Statistical methods

All statistical analyses were carried out using SPSS program, Version 13.0, and values were expressed as the mean ± SD. The comparison of data from multiple groups was made using ANOVA, from 2 groups by independent sample T test. IL-1Ra, sCRP, anti-beta2-glycoprotein, anti-cardiolipin levels were not distributed normally and so the significance of differences between levels of patients compared to controls were assessed using the Mann-Whitney test. Correlation analysis was made using Pearson's parametric and Spearman's nonparametric tests. P values less than 0.05 were considered statistically significant.

RESULTS

1. Results of the retrospective analysis of patients with SLE

Lupus nephritis was developed in 144 (26.1 %) of 551 SLE patients followed between 1974 and 2004. 81.2% of LN patients had kidney biopsy, while in the rest of the cases biopsies were not performed due to contraindications. 90% of patients were women, the mean age at the time of renal biopsy was 31.9 years, the mean duration of SLE was 11 years, while for women and 15 years for men. Occurrence of LN was 3-4 years after the onset of SLE.

1.3% (n=2) of patients had minimal mesangial and 11.1% of them (n=16) had mesangial proliferative LN, extrarenal manifestation were arthritis, pleuritis and hemocytopenias. The patients received 0.5-1 mg/kg methylprednisolon, 3 patients were administered per os cyclophosphamide (CYC) in the II. histological group and 4 patients azathioprin (AZA).

12.5% (n=18) had focal LN, 4 patients had nephritic syndrome at the beginning, the most common extrarenal symptoms were photosensitivity, Raynaud phenomenon, polyarthritis. The anti-DNA antibody level was significantly higher than in the first two groups. Besides 0.5-1 mg/kg methylprednisolon three patients received parenteral CYC (800 mg/month for half a year), maintenance therapy was 100 mg/die AZA for 7 patients. 2 patients received mycophenolate mofetil (MMF) 1 g/die after the 2^{nd} and 4^{th} relapse.

Majority of patients, 38.8% (n=56) had diffuse proliferative LN, 22 patients had nephrotic syndrome, six had hypertension and two azotaemia at the beginning. The anti-DNA antibody level was significantly higher than in other groups, except group III. Extrarenal manifestations were photosensitivity, Raynaud-phenomenon, polyarthritis, pleuritis, pericarditis, sicca-syndrome, secunder antiphospholipide syndrome and 4 patients had

neuropsychiatric symptoms. The patients received 0.5-2 mg/kg methylprednisolon, 37.5% of them (n=21) were administered 800 mg parenteral CYC, 3 patients had plasmapheresis in the acute phase. 25 patients relapsed within the first year, 9 received repeated CYC parenterally, other patients did not received CYC due to contraindications (leucopenia, infection). Two patients had chronic renal failure, one had end-stage renal disease.

13.8% of patients had membranous LN, 3 patients had nephrotic syndrome, 4 hypertension, nephritis syndrome at the beginning. Extrarenal manifestations were polyarthritis, Raynaud-phenomenon, photosensitivity, neuropsychiatric symptoms. Patients received 0.5-2 mg/kg methylprednisolon, five of them were administered 800 mg parenteral CYC, one patient had plasmapheresis in the acute phase, maintenance therapy was 100 mg/die AZA in 3, 150 mg/die cyclosporine A, and 2 patients 1g/die MMF. Two patients had chronic renal failure, and one had renal transplantation.

4.86% of patients (n=7) had histological approved glomerulosclerosis. One patient had hypertension, nephritic syndrome and one renal failure before renal biopsy. Beside methylprednisolon treatment two patients received 800 mg cyclophosphamide, and 100 mg/day azathioprine maintenance therapy.

21 patients did not have renal biopsy because of different contraindications. 4 patients were administered parenteral cyclophosphamide beside methylprednisolon (these patients clinically were same as patients with diffuse proliferative LN), 7 patients received 100 mg/day azathioprine, two patients cyclosporineA (150 mg/day), one had plasmapheresis, and one patient iv. immunoglobulin (400 mg/kg/die).

End stage renal disease developed in two patients, one patient had renal transplantation, one patient died out of this group.

2. Serum and urine cytokine measurements in patients with lupus nephritis

Serum and urinary level of cytokines were detected in 36 patients with lupus nephritis (34 female and 2 male, mean age: 43.36±11.53 years), 23 patients with SLE without renal involvement (19 women and 4 men, mean age: 54±8.71) and 30 healthy controls (23 female and 7 male, mean age: 45.5±12.4) and we analyzed the correlation with classic activity parameters. The mean duration of the disease was 7.2±4.5 in LN and 9.7±7.4 years in patients with SLE without nephritis. SLEDAI was 2.82±2.4 for LN and 2.75±2.9 for patents with SLE without renal disease.

Serum IL-1, IL-2 (both $p<0.05$), IL-6, IL-13 and IFN-γ ($p<0.001$) levels were significantly higher in lupus nephritis patients, as compared to patients with SLE without renal involvement and healthy controls. Urinary level of IL-1 and TNF-α were significantly higher in SLE patients without renal disease ($p=0.012$ and $p<0.001$), while urinary IFN-γ was significantly higher in LN patients ($p=0.002$).

Urinary IL-8 level had a positive correlation to SLEDAI ($R=0.54$, $p=0.006$), IL-1 correlated to IL-2 ($R=0.69$, $p=0.027$), IFN-γ ($R=0.76$, $p<0.001$) and urinary IFN-γ level ($R=0.98$, $p=0.004$). IL-4 level correlated to IL-10 ($R=0.64$, $p<0.001$), TNF-α ($R=0.85$, $p<0.001$), IFN-γ ($R=0.36$, $p=0.047$), urinary IL-8 ($R=0.6$, $p<0.001$) and IL-10 ($R=0.43$, $p=0.032$).

3. IL-1-receptor antagonist level measurement of patients with lupus nephritis

17 consecutively selected SLE patients (16 female, 1 male with active disease without lupus nephritis, mean age±SD: 47.58±11.9 years), 15 SLE patients (14 female, 1 man) with histologically proven lupus nephritis: 7 patients clinically inactive (IALN, all female), mean age: 38.4±11.62 years, and 8 patients (ALN, 7 female, 1 male) with lupus nephritis clinically active, mean age: 39.16±7.9 years and 10 healthy controls (5 female and 5 male, mean age: 32.4±9.5 years) were included. Clinical disease activity was measured using the SLEDAI. The values of the median SLEDAI were 4.5±3.6 for active SLE patients, 2.66±3.2 for inactive LN patients, and 6.85±1.78 for ALN ($p=0.089$). The mean complement level was significantly higher in active lupus nephritis patients. Active SLE patients had higher antiDNA level, polyarthralgia and hypocomplementaemia.

Patients with active SLE had the highest IL-1Ra level (1025±948 pg/ml) and the difference was significant as compared to all other groups and controls ($p<0.001$).
Significant correlation was found between IL-1Ra level and SLEDAI in inactive LN patients ($n=7$, $R=0.76$, $p=0.04$,). There was a positive correlation between IL-1Ra levels and beta2-glycoprotein ($n=8$, $R=0.75$, $p=0.03$) and anti-cardiolipin levels ($n=8$, $R=0.75$, $p=0.001$) in active lupus nephritic patients.

Active SLE patients had a highly significant elevation not only in the levels of IL-1Ra (1025±948 pg/ml, $n=17$ *vs.* 118.3±58.8 pg/ml $n=10$, $p<0.001$) but in CRP level (17.26±12 *vs.* 1.42±0.3, $p<0.001$, infection was excluded in these patients) compared to the healthy controls reflecting the activity of disease. There was a positive correlation between IL-1Ra and CRP levels in active SLE patients ($n=17$, $R=0.68$, $p=0.015$).

Active lupus nephritic patients received significantly higher dose of methylprednisolone: an average of 22.85±10.4 mg/day *vs.* 16±8 mg/day administered to active SLE patients, (p=0.046), while patients with inactive LN received 11.3±4.6 mg methylprednisolone (p=0.013 *vs.* active LN patients).

In the active SLE group 3 patients received 1.5 mg/kg/day cyclosporine, 4 active lupus nephritis patients were on monthly cyclophosphamide therapy (400-600 mg/month). One of patients with inactive LN received 2mg/kg/day cyclophosphamide maintenance therapy.

DISCUSSION

1. Results of the retrospective analysis of patients with SLE

Systemic lupus erythematosus is a polysystemic autoimmune disease, involvement of the kidneys and central nervous system are the most serious complications, which need early aggressive treatment. Survival of the SLE patients using new aggressive drugs have improved. The 5-year survival of these patients increased from 50% (1960) to 80% (1990) and nowadays the survival rate is 83-92%. Occurrence of lupus nephritis is a bad prognostic factor, so the early diagnosis and adequate treatment improves the survival of patients. The general clinical check-up of SLE patients is necessary.

In the retrospective study clinical and serological data has been presented from a relatively large cohort of patients with SLE. The occurrence of LN in our SLE patients was about 26.1%. In another study, Cortes-Hernandez *et al*. found LN in 30-40% of lupus patients, while others have found that 30-60% of lupus patients had anomalies of renal function or found abnormalities during urine analysis. The diffuse proliferative LN was the most frequent histological subgroup, and this percent would be higher if we include the patients without renal biopsy which clinically are similar to the diffuse proliferative form. The incidence of LN flares was found between 27-66% in different studies, whereas in our study we found it 21%. The lower rate of lupus nephritis and relapses are due regular check-ups and early, aggressive treatment.

Our patients with SLE are followed every three months or even more often if considered necessary, irrespective of clinical activity. Routine blood tests, serological markers, urine analysis were performed also the physical status was assessed at each time point. If renal involvement was suspected, kidney biopsy was performed to find out the exact

histological type of renal involvement. With this approach early, intensive therapy could be started. If the clinical condition of a patient was severe, immunosuppressive therapy was started without performing the biopsy. Furthermore, we also put stress on the treatment of proteinuria, hypertension, hypercholesterolemia, management of cardiovascular risk factors, delaying the progression of chronic kidney disease (moderating the protein sodium, and fluid intake, quit smoking), and if needed suitable treatment of the chronic kidney disease was started. As a result of the adequate care the general survival and outcome of our patients with SLE improved.

2. Serum and urine cytokine measurements in patients with lupus nephritis

Notification of lupus nephritis before organic disorder, prediction of flares, starting agressive therapy as early as possible, the follow-up of successful treatment is desirable. There is an intensive need for identifying the best biomarker for monitoring flare activity. My goal was to test possible biomarkers reflecting flares. I analyzed the serum and urinary cytokine levels of patients with LN and SLE patients without renal involvement.

In accordance to other studies IL-10 and IFN-γ levels of patients with LN was significantly higher. The data related to IL-6 level are conflicting, some authors did not find difference between serum IL-6 levels of patients with and without lupus nephritis, others find that IL-6 level correlated to activity of lupus nephritis. Our lupus nephritis patients had significantly higher serum IL-6 level, while TNF-α was significantly higher in SLE patients without renal involvement.

3. IL-1-receptor antagonist level measurement of patients with lupus nephritis

The interleukin 1 (IL-1) family of cytokines includes two major agonist molecules (IL-1α and IL-1β) with proinflammatory effects and a specific antagonist for the IL-1 receptor named interleukin 1 receptor antagonist. IL-1Ra is the natural antagonist of IL-1β. The balance between IL-1 and IL-1Ra is important in the regulation of inflammatory responses. IL-1 is produced by mesangial cells in vivo. Both secreted and intracellular forms of IL-1Ra are produced by the kidneys. In SLE patients with renal involvement an increased frequency of IL-1Ra allel 2 (IL-1RN*2) has been found, mediating a pro-inflammatory IL-1b/IL-1Ra balance. Suzuki *et al*. found that high serum level of IL-1Ra was a good indicator of disease

activity. In our patients the serum level of IL-1Ra and CRP is elevated in all SLE patients and IL-1Ra correlated with SLEDAI in inactive lupus nephritis patients. Liou *et al* have found the same correlation between IL-1Ra and CRP in untreated SLE patients, suggesting that IL-1Ra is an acute phase protein in SLE. IL-1Ra was the highest in active SLE patients without renal involvement; it was also significantly higher in active lupus nephritis patients as compared to inactive LN patients. There was no significant difference between inactive LN patients and the control group. IL-1Ra level of active LN patients was significantly higher than in inactive LN patients.

Active lupus nephritis patients received the highest dose of methylprednisolone, significantly more than active SLE or inactive lupus nephritis patients, so it follows that the higher amount of methylprednisolone could decrease IL-1Ra level. The reason for lower serum IL-1Ra concentration could be due to higher methylprednisolone dose used in this group. The prognosis of patients with higher IL-1Ra level was better and the development of end stage renal disease is lower.

In conclusion, we analyzed the serum IL-1Ra level of patients with extrarenal manifestations of SLE and patients with lupus nephritis. We found that patients with active renal disease had a significantly higher need of steroids than active patients with extrarenal manifestations, which could be due to higher level of the natural inhibitor of IL-1 observed in these patients; while lower levels of IL-1Ra in lupus nephritis patients could be due the a deficiency in IL-1Ra production. The defective production of IL-1Ra could be part of the pathogenesis of lupus nephritis. Measurement of IL-1Ra level in SLE patients could help to predict future renal involvment.

SUMMARY, CONCLUSIONS

1. Occurrence of lupus nephritis in our SLE patients was 26.1%, the most frequent histological type was diffuse proliferative glomerulonephritis (38.8%), which would be higher if we add those patients who are clinically similar to patients with diffuse proliferative LN but did not have renal biopsy due to different contraindications. The rate of relapse within our patients was lower than found by others, only 21%. Anti-DNA level was significantly higher in proliferative forms. 76% of patients with diffuse proliferative lupus nephritis received parenteral cyclophosphamide.
2. Serum IL-6 concentration of patients with lupus nephritis was significantly higher than in patients without nephritis and controls, which could confirm the role of iL-6 as a biomarker in lupus nephritis. Moreover IL-10 and IFN-γ level of patients with LN was significantly higher. While TNF-α and IL-1 serum and urinary level was significantly higher in patients with SLE without renal involvement. The highest levels of IFN-γ and TNF-α were found in diffuse proliferative LN.
3. Serum IL-1Ra level of active SLE and lupus nephritis was significantly higher as compared to control group while, the serum IL-1Ra level of active SLE patients without renal disease was significantly higher than of patients with LN. The serum IL-1Ra level correlated to sCRP level. IL-1Ra level of patients with inactive LN correlated to SLEDAI. Measurement of serum IL-1Ra could predict renal involvement of SLE patients.

PUBLICATIONS

Articles that directly grounded the PhD theses:

1. **B. Brugos,** E. Kiss, P. Szodoray, G. Szegedi, M. Zeher: Retrospective analysis of patients with lupus nephritis, data from a large clinical immunological center, Scand J Immunol. 2006 Oct;64(4):433-437. **IF: 2.1**
2. **B. Brugos**, E. Kiss, C. Dul, W Gubisch, G. Szegedi, S. Sipka, M. Zeher: Measurement of interleukin-1 antagonist in patients with systemic lupus erythematosus could predict renal manifestation of the disease. Hum Immunol. 2010 Sep;71(9):874-877. **IF: 2.55**
3. **Brúgós B**., Zeher M.: Biomarkerek jelentősége lupus nephritisben. Orv Hetil. 2010 Jul 18;151(29):1171-1176.

Other articles

1. Balogh Z., Bacso J., **Brúgós B**., Paragh Gy.: A haj szelén tartalmának koncentrációja hypercholesterinaemiás betegekben. Magyar Belorvosi Archivum, 3/2000 (209-213).
2. Kiss E., Buda B., Tarr T., **Brúgós B**., Lakos G., Szegedi J., Szegedi Gy.: Cardiovasculáris szövődmények és azok kockázati tényezőinek előfordulása szisztémás lupus erythematosusban. Allergológia és Klinikai Immunológia 2: 52, 2002.
3. **B. Brugos**, G. Hochhaus: Metabolism of dynorphin A(1-13). Pharmazie, 2004 May; 59(5): 339-343. **IF: 0.78**
4. S. Al-Fayoumi, **B. Brugos**, V. Arya, E. Mulder, B. Eppler, AP. Mauderli, G. Hochhaus: Identification of stabilized dynorphin derivatives for suppressing tolerance in morphine-dependent rats. Pharm. Res. Vol. 21, No. 8, Aug. 2004, 1450-1456. **IF: 3.93**
5. **B. Brugos**, V. Arya, G. Hochhaus: Stabilized dynorphin derivatives for modulating antinociceptive activity in morphine-dependent rats. AAPS Journal, 2004 Dec. 28, 6(4), e36. **IF: 3.54**

6. V. Arya, I. Coowanitwong, **B. Brugos**, WS. Kim, R. Singh, G. Hochhaus: Pulmonary targeting of sustained release formulation of budesonide in neonatal rats. J Drug Targeting, 2006 Dec, 14(10), 680-686. **IF: 2.77**
7. Z. Vincze, **B. Brugos**: Does impairment of renal and hepatic function influence the metabolism of thrombolytics in patinets with myocardial infarction? Pharmazie. 2008 Mar;63(3):245-246. **IF: 0.78**
8. Vincze Z., **Brúgós B**.: Befolyásolja-e a krónikus sztatinszedés a myocardialis infarktus okozta mortalitást? Appendix, É-Kelet Magyarországi Orvosi Szemle, 2008, IX. évf. 1-2 szám, 16-21.
9. Z. Vincze, C. Duncea, **B. Brugos**, D. Radulescu: In hospital mortality of patients with acute myocardial infarction in Debrecen, between 2001-2003, in correlation with the clinical signs, risk factors and comorbidities. Clujul Medical, 2009, Vol LXXXII, 55-61.

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