Cardiovascular biomarkers in diabetic nephropathy

PhD thesis

Emília Mácsai MD.

Semmelweis University Budapest

Clinical Medicine School of Ph.D. Studies

Tutor: Barna Vásárhelyi M.D., Ph.D., D.Sc.

Official reviewers: Péter Hamar M.D., Ph.D., D.Sc.

Tamás Szelestei M.D., Ph.D.

Head of the Final Examination Comittee:
Anikó Somogyi M.D., Ph.D., D.Sc.

Members of the Final Examination Comittee:
György Deák M.D., C.Sc.
László Wágner M.D., Ph.D.

Budapest

2013

Introduction

In recent years the concept of cardiometabolic and cardiorenal syndromes was outlined. The strong correlation between type 2 diabetes (T2DM), cardiovascular diseases (including heart disease), and chronic renal failure was also increasingly recognized. Today the multidisciplinary approach is emphasized in care of T2DM population.
The development of diabetic nephropathy and cardiovascular diseases with significant vascular damage share several common elements including oxidative stress, endothelial injury and inflammation. Measurement of blood or tissue biomarkers of these processes may provide an efficient tool for early diagnosis and therpeutic decision making. The aim of my PhD studies was to investigate some of these biomarkers in T2DM population complicated with nephropathy.
The natriuretic peptides are produced by heart muscle cells under pressure or volume overload. In case of progressive chronic renal failure NT-proBNP can be used as cardiac biomarker. The cardiac troponins enter into the circulation in case of myocardiac injury. In asymptomatic patients with renal failure serum cTnT are frequently elevated due to subclinical cellular lysis or direct damaging effect of uremic toxins. Both cardiac biomarkers are related to cardiovascular mortality in dialysis patients.

The role of inflammatory processes in the development of atherosclerotic plaques is outstandingly important. The

hsCRP levels are used for the characterization of systemic inflammation. Moreover, other cytokines are also used as biomarkers of inflammation: IL-6 and IL-10 are considered as proinflammatory and anti-inflammatory cytokines, respectively. The monocyte / macrophage cells are present in all stages of atherosclerosis, they have a key role in the progression of mild atheromatic lesions into complex lesions. The MCP-1 chemokine induce the subendothelial accumulation of monocytes.

Conventional glycemic markers (like hemoglobin A1C) are frequently unsuitable for the assessment of the carbohydrate metabolism in end-stage kidney disease in diabetic patients. In the past few years a non-invasive, rapid and reliable tool for the measurement of cumulative skin tissue glycation end products became commercially available. The so-called skin autofluorescence (SAF), which indicates deposition of fluorescencing analites is an accepted marker predicting mortality. However, the SAF is still not used in clinical practice widely. It is also not known what type of molecules are responsible for advanced glycation end product (AGE)-level anomalies indicated by SAF-measurement.

Several drugs used in standard therapy of diabetic nephropathy may have a favorable add-on effect on cardiovascular risk markers. These effects are only partially characterized. During my PhD work I analysed the effect of statins on cardiac biomarkers; the immunomodulatory properties of the antihypertensive agent doxazosin; and the metabolic effects of allopurinol, given to reduce serum uric acid levels.

Objectives

My aim was to collect data on cardiovascular biomarkers in patients with diabetes and kidney failure and to examine the supposed relationships with several drugs.

Cardiac and inflammatory biomarkers

Serum levels of NT-proBNP, cTnT, hsCRP, IL-6, IL-10 and MCP-1 were measured in hemodialysed diabetic and non-diabetic patients. I investigated the correlation of tested parameters with clinical state, blood pressure, bioimpedance data, and diabetic complications.

Skin autofluorescence in renal insufficiency and diabetic patients

Skin autofluorescence (SAF) values, clinical parameters, and their relationship with therapy were assessed in peritoneal dialysis patients. In diabetic adolescents I examined how some skin AGE levels associate with SAF.

Modification of cardiovascular biomarkers by known therapeutic agents

Three common drugs (doxazosin, fluvastatin and allopurinol) used for medical therapy of diabetic population were evaluated if they exert an add-on effect on cardiac biomarkers, immune phenotype and metabolic status in T2DM patients.

Patients and methods

Patients

A cross-sectional survey was carried out with 28 and 40 diabetic and non-diabetic hemodialysed patients in stable condition for at least 3 months. Cardiac biomarkers including NT-proBNP and cTnT levels, and hsCRP, IL-6 and IL-10 and MCP-1 levels were measured. The results were related to patients' clinical characteristics.

The SAF test study included 198 patients on chronic peritoneal dialysis; the prevalence of DM was 47% in this population.
SAF values were related to 5 different AGEs measured with DESI-MS technique in 16 T1DM adolescent patients.
The effect of fluvastatin on cardiac biomarkers were evaluated in 42 diabetic subjects.
The T cell phenotype changes upon doxazosin therapy were measured in 10 T2DM men.
The association between allopurinol therapy and HbA1c levels were followed in 132 diabetic patients.

Applied methods

Bioimpedance assessment

The body composition was measured with bioimpedance analysis (BIA), InBody 3.0 Biospace multifrequency segmental 8-electrode device was used. The measurements were made at standing position, the ecv/ twv ratio was used as volume marker. The total body fat

content (fat mass%), and BMI was simultaneously recorded.

Clinical and laboratory data

The patients' clinical data and laboratory results were collected by reviewing the medical documentation.

Echocardiographic examination

Aloka 5505 echocardiographic machine was used, 2D method according the registered standards. To calculate the left ventricular mass index the formula of Devereux-Reichek, and Gehan-George was applied. {BSA-m^2=0,0235x (body weight in kg) ^{0,51456}x (height in cm)^{0,42246}}

Skin autofluorescence measurement

The AGE Reader device (DiagnOptics, Groningen, The Netherlands) in the range of 300-600 nm was used for measurement.

DESI-MS measurements The patients' fingertip was cleansed by distilled water prior to desorption electron spray ionization mass spectrometry (DESI-MS) measurements. We used modified OmniSpray ion source, which was coupled with two spectrometer: TSQ Quantum triple quadrupole mass spectrometer and LTQ Orbitrap Discovery Fourier transform mass spectrometer.

Flow cytometric analysis

Mononuclear cells of peripheral blood were separated from whole peripheral blood by Ficoll-Paque Plus

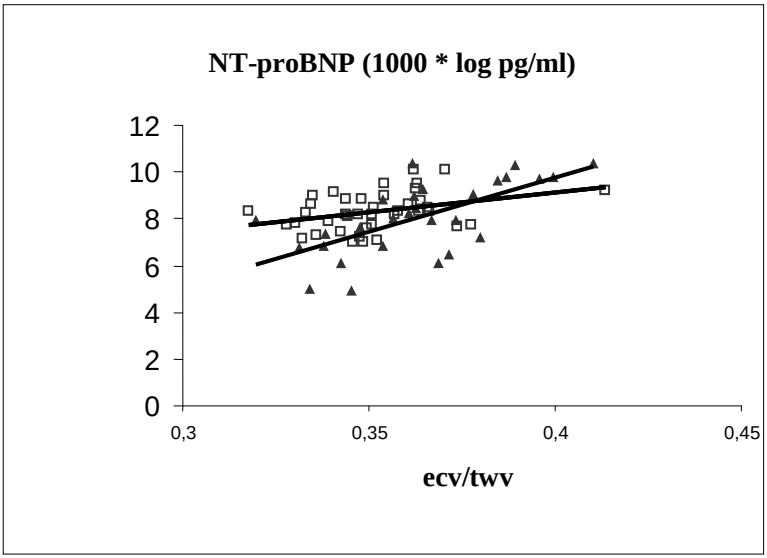
gradient system. The prevalence of FoxP3 positive (Treg) cells and CD4, CD8, CD25 and CD 69 positive cells were tested with appropriate dyes and antibodies with BD FACS Aria system.

Statistical methods

The effect of independent factors on certain biomarkers was analysed by ANOVA. In patient groups data were compared with the Mann-Whitney test. Correlations were tested with Pearson correlation test and Spearman rank test in case of normally and non-normally distributed variables, respectively.

Results

In diabetic patients, hyperhydration correlated with NT-proBNP levels (28 diabetic patients: r = 0.66, p = 0.001; 40 non-diabetic patients: r = 0.35, p = 0.034)



The NT-proBNP levels are linked to the volume overload expressed in ECV/tw; diabetic (▲) and non-diabetic (□) patient populations; (ECV / twv: extracellular water volume/ volume of total body water as measured by BIA)

We could not find relationship between cardiac biomarker cTnT and non-cardiac complications of diabetes, medication, sex, age, time spent on dialysis, traditional and novel risk factors, HbA1c and duration of diabetes.

In hemodialysed diabetic patients the mean EPO dose ($p = 0.03$), total cholesterol ($p = 0.039$) and BMI ($p = 0.043$) showed a close relationship with hsCRP. In the non-diabetic group a significant correlation ($p = 0.025$) between the serum fibrinogen and hsCRP levels was observed. The diabetic patients had greater BMI ($p < 0.00001$) and % body fat mass ($p = 0.004$) than non-diabetic patients on hemodialysis.

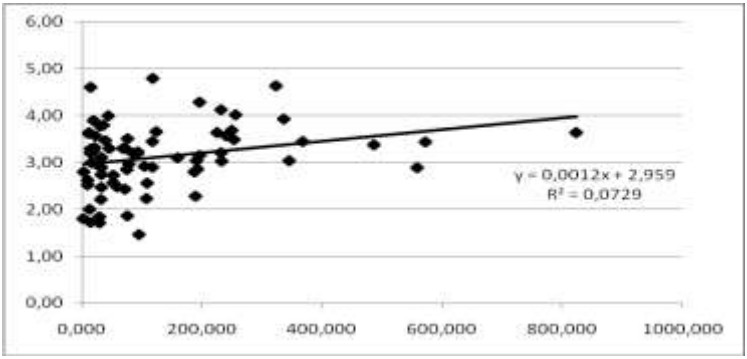
The IL-6 levels ($p = 0.02$) and IL-10 levels ($p = 0.003$) were higher in the diabetic compared with non-diabetic hemodialysis patients. They also had elevated levels of fibrinogen ($p = 0.02$), while prealbumin levels were almost significantly lower ($p = 0.054$).

In total (n=42) diabetic patients cared at outpatient department urinary albumin excretion and serum MCP level correlated proportionally, the correlation maintained ($p = 0,02$) after adjustment to CRP, HbA1c, BMI, LDL, and serum uric acid values. Serum levels of MCP-1 and CRP did not correlate with diabetic complications.

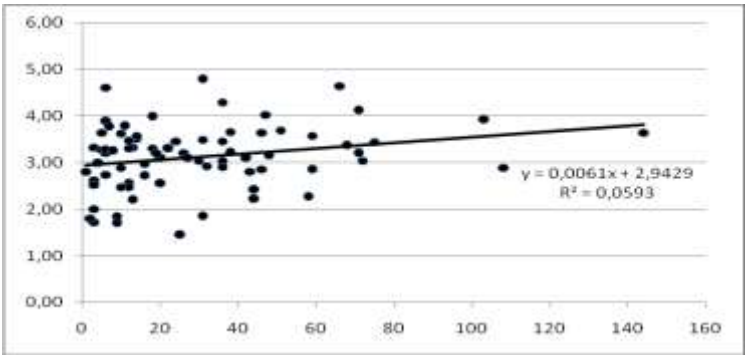
In peritoneal dialysis patients ($n = 198$) age ($p=0.15$), diabetes ($p = 0.012$), and the icodextrin treatment ($p = 0.005$) were significant independent predictors of SAF. Patients on icodextrin had larger peritoneal ultrafiltration, and smaller residual diuresis and clearance ($p <0.001$). In this patient subgroup the prevalence of diabetes mellitus was higher (60% v. 40%), and duration of diabetes was longer ($p = 0.01$), and the patients were younger ($p =$

0,02). Intraperitoneal cumulative glucose exposure was greater (p = 0.05), and these patients had higher hsCRP (p = 0.03), and lower serum triglycerides, total cholesterol (p = 0, 04), and serum uric acid levels (p = 0.01). Skin autofluorescence was higher in the icodextrin group (p = 0.02) compared to patients on traditional regime.

In non-diabetic and non-icodextrin subgroup (n = 76) the cumulative glucose exposure and PD duration time correlated with SAF value.



X-axis: glucose exposure (kg) Y-axis: SAF (arbitrary unit)
Correlation (n = 76) between the SAF and the cumulative intraperitoneal glucose exposure was resulted by nonparametric Spearman test: r = 0.2941; p = 0.011.



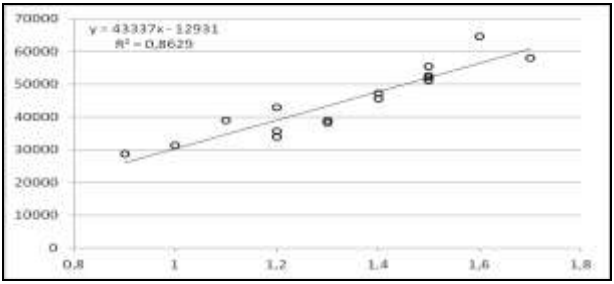
X-axis: the PD duration time (months), Y-axis: SAF (arbitrary unit)
SAF and PD duration time was correlated ($r = 0.25$; $p = 0.03$).

SAF and some AGE-molecules (glucuronic acid, 3-indoxyl sulfate, 3-hydroxybutyrate, phenol sulphate, and pentosidin) measured by DESI-MS correlated significantly ($p < 0.0001$).

The Pearson correlation between the results of the SAF and analytes measured by DESI-MS

	correlation coefficient (r)	95% CI	p value	DESI-MS value (arbitrary unit)
glucuronic acid	0.91	0.77 – 0.97	p< 0.0001	35789±8012
3-indoxyl-sulfate	0.86	0.64 – 0.95	p< 0.0001	1054±247
3-hydroxy-butirate	0.91	0.76 – 0.97	p< 0.0001	6181±1666
phenol-sulfate	0.87	0.67 – 0.95	p< 0.0001	542±166
pentosidin	0.90	0.74 – 0.96	p< 0.0001	1192±395

The 5 AGE products' summed spectral values correlate with the SAF (n = 16)



In patients on statins NT-proBNP (p = 0.009) and cTnT (p = 0.02) levels were lower than in those without statins.

In patients taking doxazosin for 3 months morning hypertension, HbA1C, total cholesterol and LDL cholesterol levels and also microalbuminuria decreased significantly. T cell phenotype also changed with an increase in $CD4^+$ $FoxP3^+$ / $CD4^+$ (p = 0.009) and $CD4^+$ $CD25^{high}$/$CD4^+$ (p = 0.001) cell ratio and statistically significant decreases in the $CD4^+$ $CD69^+$ / $CD4^+$ (p = 0.003) and $CD8^+$ $CD69^+$ / $CD8^+$ (p = 0.022) cell ratios.

Serum uric acid levels decreased in patients with diabetes starting allopurinol. The average HbA1C levels were lower at the time of the second visit. The results of the multiregression analysis showed that allopurinol treatment at each time point was associated with lower A1C values p <0.0001).

Conclusions

Cardiac and inflammatory biomarkers in hemodialysis and factors affecting the level of early-stage nephropathy patients

Cardiac biomarker NT-proBNP in both diabetic and non-diabetic hemodialysis patients is linked to the extracellular / total body water ratio, the connection is stronger in the diabetes group. The cTnT in hemodialysis patients mainly refers to the myocardial cell damage. The hsCRP, a marker of inflammation in these patients correlated in diabetic patients with erythropoietin dose, total cholesterol and BMI, and in non-diabetic patients with the serum fibrinogen level. In hemodialysis population IL-6 and IL-10 levels were higher in presence of diabetes, their relationship with inflammatory processes is supported by their association with serum fibrinogen and prealbumin levels. In the initial stages of diabetic nephropathy serum MCP-1 levels correlate with the degree of albuminuria.

Skin autofluorescence in renal insufficiency and diabetic patients

AGE accumulation in the skin is significantly affected by age, previous diabetes, and current use of icodextrin treatment in peritoneal dialysis patients. Diabetic adolescents have shown that the SAF values are closely linked with the levels of some advanced glycation end products.

Therapeutic effects in patients with diabetes on cardiac biomarkers, immune phenotype and metabolic status

Among cardiovascular markers of diabetic patients the NT-proBNP and cTnT levels were lower if they were on fluvastatin. T2DM patients with treated by alpha-1-inhibitor doxazosin showed immunomodulatory effect. In T2DM patients with poor metabolic control and hyperuricemia allopurinol therapy decreased the levels of HbA1C.

Publications related to PhD theses

1. Mácsai E.; Vásárhelyi B.; Madácsy L. (2005) Újabb adatok a diabéteszes nefropátia molekuláris patogenezisének kutatásában. MBA (58.): 91-97.
2. Mácsai E.; Széll J.; Ladányi E.; Treszl A.; Vásárhelyi B.; Madácsy L. (2007) Kardiális biomarkereket meghatározó tényezők vizsgálata diabéteszes és nem diabéteszes hemodializált betegekben. Orv Hetil (11): 483-488.
3. Mácsai E.; Mizsik T.; Fodor B.; Ladányi E.; Treszl A.; Vásárhelyi B. (2007) Kardiális biomarkerek szintjét befolyásoló tényezők hemodializált diabéteszes betegekben : a fluvastatin hatása MBA (62.): 149-153.
4. Mácsai E.; Fodor B.; Fekete A.; Treszl A.; Vásárhelyi B.; Madácsy L. (2007) Befolyásolja-e a diabétesz hemodializált betegekben a citokin-szinteket? Hypert és Nephrol. (11.): 21-26.
5. Mácsai E.; Fodor B. (2008) Van-e kapcsolat a szérum MCP-1 és a dokumentált diabéteszes szövődmények között diabetológiai ambulancián gondozott betegekben? Hypert és Nephrol. 12 (5):184-188.
6. Mácsai E.; Cseh Á.; Budai G.; Mészáros G.; Vásárhelyi B.; Fischer K.; Szabó A.; Treszl A. (2009) Effect of 3 months of doxazosin therapy on T-cell subsets in type 2 diabetic patients J Int Med Res 37(6):1982-1987. **IF: 0,90**
7. Mácsai E.; Vásárhelyi B.; Madácsy L. (2010) A kardiovaszkuláris morbiditást befolyásoló

tényezők diabéteszes nefropátiában. Metabolizmus (2): 90-92.
8. Mácsai E; Joó I, Pavelka R, Eisenbergerné K.A, Tódorné R.M, Vásárhelyi B, Madácsy L. (2011) Az allopurinol kezelés helye a kardiometabolikus rizikó csökkentésében Metabolizmus 9(3): 37-40.
9. Mácsai E., Benke A., Cseh Á., Vásárhelyi B. (2012) Factors influencing skin autofluorescence of patients with peritoneal dialysis Acta Physiol Hung 99(2):216-222. **IF:0,82**
10. Mácsai E., Tölgyesi K., Benke A.(2012) A diabetes mellitus és a peritoneális dialízis kapcsolata LAM 22(02): 129-133.
11. Mácsai E. (2012) A bőr autofluoreszcenciájának mérése a diabetológiai és klinikai gyakorlatban Orv Hetil 153(42):1651-1657.
12. Mácsai E., Takáts Z., Derzbach L., Körner A., Vásárhelyi B. (2013) Verification of skin autofluorescence values by mass spectrometry in type-1 diabetic adolescents Diabetes Technol Ther 15(3):269-72. **IF 1,93**

Publications not related to PhD theses

13. Mácsai E; Szabó E; Király M; Benke A; Lakatos L. (2011) A HbA1c-szint összehasonlítása nem diabeteses és diabeteses betegcsoportok között nefrológiai ambulancián Hypert és Nephrol 15(2):67-73.
14. Mácsai E. (2012) A diabetes mellitus kezelésének speciális szempontjai idős korban LAM 22(03):205-209.
15. Mácsai E., Vizeliné S. K., Tölgyesi K., Benke A. (2012) Hogyan kerülhetjük el a hypoglykaemiát hemodializált diabeteses betegek inzulinkezelése során? LAM 22(6-7):429-433.
16. Mácsai E., Benke A., Gergely L., Vándorfi Gy. (2012) A szénhidrát anyagcsere korrekciója előrehaladott veseelégtelenségben Diab Hung 20(4):256-262.
17. Toldi G.; Bekő G.; Kádár G.; Mácsai E.; Kovács L.; Vásárhelyi B.; Balog A. (2012) Soluble urokinase plasminogen activator receptor (suPAR) in the assessment of inflammatory activity of rheumatoid arthritis patients in remission. Clin Chem Lab Med. 51(2):327-32. **IF: 2,15**

Acknowledgements

First of all I want to thank my supervisor, Professor Barna Vasarhelyi, who mentored my scientific work since 2005. I wish to thank Prof. Laszlo Madácsy who supported my research activities and plans. I would like to thank the staff, PhD students and assistants at Research Group of Pediatrics at Semmelweis University for their support in carrying out the measurements.

InBody 3.0 Biospace device and apparatus was provided by Fresenius. I would like to express my thank to Erzsebet Ladanyi Medical Director and Gyorgy Zoltan President Director. I thank for hemodialysis patients' echocardiographic parameters for Anikó Csótay, MD. Cytokine measurements was performed at the 3rd Department of Internal Medicine, University of Debrecen, Professor Sandor Sipka and Gabriella Lakos. Bertalan Fodor MD PhD. also provided help and friendly support.

In studies involving patients on peritoneal dialysis Peter Horn Managing Director and Attila Benke, MD Medical Director provided support. The operative help in measurements of Katalin Tölgyesi senior nurse and BRAUN Dialysisnetwork PD teams is also highly appreciated.