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Molecular markers with impact on kidney graft survival and glomerulopathies progression

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CONTENT

ABSTRACT

The progression of chronic glomerulopathy and graft rejection is affected by a number of proinflammatory cytokines, whose role in the pathogenesis of damage is poorly understood. The aim of this dissertation was to identify reliable risk markers of renal dysfunction progression and thereby contribute to a more effective patient treatment.

Human native kidney biopsies with histologically confirmed diagnosis of glomerulopathy or kidney graft biopsies were analysed. Intrarenal gene expressions were measured by RT-qPCR. Single nucleotide polymorphisms were detected by methods based on PCR-RFLP. Immunohistochemical staining was used to identify and quantify the mononuclear cell infiltration.

Gene expression of *TGF-β1, HGF, BMP7, MCP-1, RANTES* and mononuclear cell infiltration were associated with poor renal function and proteinuria at the time of IgA nephropathy diagnosis. Progression of IgA nephropathy during the 2-year follow-up was shown to be dependent on the degree of chronic vasculopathy and *TGF-β1* expression in the kidney.

Patients with graft dysfunction and enhanced intrarenal expression of *TGF-β1*, *MCP-1* had significantly shorter graft survival. Higher mRNA expression of *IL-10, TGF-β 1, IL-6, MCP-1, RANTES* and *TNF-α* was observed in patients with graft dysfunction presented at the time of biopsy. Correlation between the degree of allograft tubulitis and *MCP-1, RANTES* and *TGF-β1* gene expression was found. None of the examined functionally relevant gene polymorphisms increased the risk of subclinical rejection, acute rejection or allograft nephropathy in selected patients.

In conclusion, this study helps to find new biomarkers of IgA nephropathy progression and complications after kidney transplantation and thereby identify patients with increased risk.

Key words: IgA nephropathy, graft dysfunction, gene expression, gene polymorphism

INTRODUCTION

About 10 % of population suffers from kidney disease In most patients, kidney disease is imunopathogenic and leads to irreversible kidney failure. The most frequent procuring causes of kidney failure are chronic kidney glomerulopathies. The most frequent one is Immunoglobulin A nephropathy (IgAN). The IgAN is immunocomlex glomerulonephritis with poorly understood pathogenesis. It remains a problem to be able to decide the immunological activity of the disease when it can persist over decades without clinical manifestation. Predictors for poor outcome in IgAN patients are both clinical and histological ones – all of them may be more markers of irreversible damage than the activity of the disease. Recently, it has been hypothesized that molecular phenotypes of various diseases with broad forms of the manifestation, such as IgAN, might allow for discerning patients at risk for disease progression and accordingly personalizing their treatment [1-4]. The selection of candidate genes is based on experimental models. These include transforming growth factor-beta 1 (TGF-β1) [5, 6], hepatocyte growth factor (HGF) [7], bone morphogenic protein-7 (BMP7) [8], monocyte chemotactic protein-1 (MCP-1, known also as CCL2) and regulated upon activation normal T-cell expressed and secreted (RANTES, known also as CCL5)[9].

Similarly, the role of immune cells infiltration has been hypothesized in the progression of chronic kidney diseases including

IgAN as well. Immunohistochemical evaluation of tubulointerstitial inflammation was shown in a retrospective study to be a useful tool in determining the prognosis in IgAN and CD3 positive cells infiltration and strongly correlates with interstitial fibrosis and progression of IgAN [10].

One of the aims of this dissertation was to evaluate the prognostic role of the molecular phenotype, immune cells infiltration, clinical and histological features in IgAN patients.

The best method of therapy for patients with end-stage renal disease is kidney transplantation. Compared to treatment with dialysis, patients after kidney transplantation have a higher quality of life and lower morbidity and mortality rate. While at the beginning of kidney transplantation the primary problem was to overcome acute rejections and infections, in the past 15 years it has focused more on long term graft survival and the side effects of immunosuppressive treatment. The introduction of Cyclosporin A as an immunosuppressive in 1980´s increased the one year graft survival rate of 10-20%, but the long term outcome did not change [11]. CAN, reclassified as interstitial fibrosis and tubular atrophy (IF/TA) [12], is the most common cause of kidney graft failure [13-16]. Molecular and immunological mechanisms of CAN development are not quite clearly understood.

In experimental models the ones to play a role in CAN development were TGF-β1, tumour necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), interlukins 6 and 10 (IL-6, IL-10) and chemokines RANTES

(CCL5), MCP-1 (CCL2), their receptors CCR2 and CCR5 and others. Thus one aim of this submitted dissertation was to evaluate the gene expression in the biopsies of kidney transplant patients with CAN.

The other aim of this submitted dissertation was to find the influence of polymorphisms in selected genes (*TNF-α*-308G/A, *MCP-1*-2518A/G, *RANTES*-403G/A, -109T/C, -28C/G, CCR2+190G/A, *IFN-γ*+874A/T, *TGF-β1*-869T/C, +915G/C a *CCR5*Δ32) on kidney graft outcome by studying a large cohort of well-characterized patients who underwent renal transplantation.

AIMS

1. The first aim of this dissertation was to evaluate the prognostic role of the molecular phenotype, immune cells infiltration and clinical and histological features in IgAN patients.
2. The second aim of this submitted dissertation was to evaluate the gene expression in the biopsies of kidney transplant patients with CAN.
3. The third aim of this submitted dissertation was to find the influence of polymorphisms in selected genes on kidney graft outcome.

PATIENTS AND METHODS

Patients

Fifty-one patients in whom IgAN was histologically diagnosed between January 2005 and May 2007 were prospectively enrolled in the

study and followed for 24 months. 68.6 % were males; the median age at the time of biopsy was 39.1 [32.1 – 52.5] years. The following clinical variables were recorded from the patient: serum creatinine, glomerular filtration estimated by MDRD formula, proteinuria, erythrocyturia, serum IgA, blood pressure, cholesterol and triglycerides. None of our cohort had received immunosuppression before kidney biopsy.

Based on the disease progression during the 24-month follow-up after kidney biopsy ten patients were classified as "progressors" in whom serum creatinine at the biopsy increased for more than 25% of baseline values (n=4) or who had renal failure during the 2 years follow-up (n=6). Patients in whom serum creatinine decreased during the 24-month follow-up or remained stable within normal range were classified as "non-progressors" (n=37). Four patients in whom the follow-up was not available were excluded from the analysis.

For the purpose of transplant study, we used 74 biopsies that revealed either CAN according to the Banff 97 classification or normal morphological findings. Biopsies were performed between November 2001 and June 2003 because of late graft function deterioration (n = 47) or according to protocol (n = 27). There were 49 males and 25 females; the mean age at the time of biopsy was 47.0 ± 12.6 years. Cyclosporine A was the cornerstone immunosuppressant in 58 of them, tacrolimus in the rest. The renal function of patients was monitored up to 42 months after renal biopsy.

All patients gave their written informed consent to participate in the study and the Ethics Committee of the Institute for Clinical and Experimental Medicine in Prague approved the study protocol.

436 patients were also included into the study, who underwent kidney transplantation at IKEM between 1999 and 2004 and gave their consent to use their DNA for analysis of gene polymorphisms. In the post-transplant course, all patients received either cyclosporine or tacrolimus along with mycophenolate mofetil and steroids, recipients with PRA >50% received prophylaxis by muromonab-OKT3 or anti-thymocyte globulin. All AR according to Banff 97 criteria [15] episodes were biopsy proven and borderline changes were included.

Clinical and laboratory data were collected on the date that the protocol 12-month biopsy was performed. A complete physical examination was undertaken for all patients. Laboratory evaluation included creatinine, total cholesterol and total triglycerides. The glomerular filtration rate was estimated by the Cockcroft-Gault formula [17].

Renal biopsies and histomorphology

All biopsies were performed using a 14-gauge Tru-Cut needle (Uni-Cut Nadeln, Angiomed, Germany) guided by ultrasound (Toshiba, Power Vision 6000, Japan). Small portions (~ 2 mm) of renal tissue from the cortical or juxtamedullary zone were immediately stored in preserve solution (RNA later, Qiagen) for expression analysis, while the majority of renal tissue taken by core biopsy was used for routine histology

performed by the standard method. Samples were routinely stained with hematoxylin and eosin, periodic acid-Schiff (PAS), aldehyde-fuchsin orange G (AFOG), Sirius red with elastic stain and periodic acid silver-methenamine (PASM). Histological examination was performed and revisited according to recent classification of IgAN (14). Biopsy tissues from allografts were scored on the basis of the Banff 97 working classification [15]. Subclinical rejection (SR) was defined as histological findings of AR including borderline changes at 12 month protocol biopsy and stable kidney graft function.

Immunohistochemistry

Immunohistochemistry was performed on 4-μm-thick paraffin sections. The slides were deparaffinized in xylene and rehydrated in graded enthanols. After deparaffinization and rehydration, the slides were heated in a microwave oven for target retrieval. Endogenous peroxidase was blocked by 0.3% H2O2 in 70% methanol for 30 minutes.

Mononuclear cells

The slides were incubated with the primary antibody (CD3 polyclonal antibody from DAKO, Denmark; CD4 and CD8 monoclonal antibody from VECTOR laboratories, Burlingame, CA; CD20 and CD68 monoclonal antibodies from DAKO, Denmark) and detection of monoclonal antibodies was performed using Histofine Simple Stain MAX PO (Nichirei, Japan). Finally, the specimens were stained with 3,3

diaminobenzidine (DAKO, Denmark) and were counterstained with Harris´s hematoxylin and embedded in Entellan (both from Merck, Germany). The number of positive cells per 1mm² was calculated using Olympus DP-SOFT (Software Imaging Systems, Münster, Germany).

Transforming growth factor β1

The tissues were preincubated with a 10% horse serum (Vector laboratories, Burlingame, CA) for 20 min. Primary antibody (anti TGF-β1, clone TB21, Abcam) was applied for 30 minutes, diluted 100x. Detection of monoclonal antibody was done using a biotinylated horse anti mouse IgG (H+L) (Vector laboratories, Burlingame, CA) diluted 200x for 30 min. Then specimens were incubated with R.T.U. Vectastain Elite ABC Reagent (Vector Laboratories, Burlingame, CA) for 30 min. Finally, specimens were stained with 3,3 diaminobenzidine (DAKO, Denmark) for 3 min and were counterstained with Harris´s hematoxylin before they were embedded in Entellan (both from Merck,Germany).

RNA isolation and RT-qPCR

After renal tissue was homogenized and total RNA extracted using RNA Blue (Top-Bio s.r.o. Czech Republic), it was reversely transcribed into complementary DNA (cDNA), as described elsewhere [18] and then the cytokine expression profile in renal tissue was analyzed. Messenger RNA of chemokines and cytokines was quantified by real-time polymerase chain reaction (RT- qPCR) (Applied Biosystems 7900HT

Fast Real-Time PCR System) using commercial TaqMan fluorogenic probes. Specific gene expression was calculated relative to the housekeeping gene HPRT (hypoxanthin-guanin-phosphoribosyl-transferase) using a comparative threshold cycle method. The plates also contain the calibrator sample for relative quantification ($2^{-\Delta\Delta Ct}$ method). All investigated mRNAs were measure in duplicates for each sample. The samples were considered negative if the Ct values exceeded 40 cycles.

Genotyping of polymorphisms

The genomic DNA was isolated from whole blood samples using a commercial kit (Whole blood DNA purification kit; Fermentas, Canada). Single nucleotide polymorphisms were determined by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism analysis (RFLP): *RANTES*, *MCP-1*, *CCR2* [19-22] or by sequence specific priming (PCR-SSP): *TNF-α*, *IFN-γ* and *TGF-β1* [23-25]. The insertion-deletion 32bp polymorphism in CCR5 was determined by a simple PCR method [26].

After testing for Hardy-Weinberg equilibrium, allele frequencies were checked for consistency with data from CEU population of European ancestry from the HapMap database [27].

Statistics

The relationship between clinical values and mRNA expression were assessed using Spearman's correlation coefficient. Differences in mRNA expression or clinical parameters between groups were analyzed using the Mann–Whitney test. The area below the receiver operating characteristic (ROC) curve and multivariate stepwise logistic regression were performed to estimate the risk for disease progression related with different transcripts, clinical values and mononuclear cell infiltration. This curve was also used for setting the cutoff points of intrarenal mRNA expression levels of studied genes with the best combination of sensitivity and specificity that indicated the renal graft dysfunction within 42 months after biopsy. Renal outcome was also assessed by Kaplan-Meier survival analysis with log-rank testing. In case of non-Gaussian distribution of values the logarithmic transformation was used. A p-value < 0.05 was considered to be statistically significant.

The Hardy-Weinberg equilibrium of alleles at individual loci was evaluated using the chi-square test. To evaluate the effect of continuous variables on the incidence of acute rejection, CAN or subclinical rejection 1 year after kidney transplantation, one-way ANOVA was used. Single-locus association analyses were performed by univariate logistic regression analysis using SPSS version 14.0 (SPSS inc., Chicago, IL, USA). Subsequently, multivariate regression adjusted for total number of mismatches, mismatches in DR locus and immunosuppression regimen was performed. For haplotype analysis,

univariate logistic regression was used. To test the effect of the polymorphisms on the graft survival, the Kaplan-Meier analysis was used.

RESULTS

IgA nephropathy (Mb. Berger, IgAN)

Gene expression, mononuclear infiltration and renal function

The gene expression patterns of *MCP-1*, *RANTES*, *HGF*, *BMP7*, *TGFβ1* and interstitial infiltrate of T cells, cytotoxic T cells, B cells and macrophages were significantly related to GFR and serum creatinine at the time of biopsy. There was no relation between analyzed parameters and T helper cell infiltration (CD4+).

Proteinuria significantly correlated with the interstitial *MCP-1* and *HGF* mRNA expression ($p<0.01$, $r=0.37$; $p<0.01$, $r=0.41$, respectively) and negatively correlated with macrophages infiltration in the glomeruli ($p<0.05$, $r= 0.36$).

Association of interstitial infiltration with the gene expression

The interstitial infiltration of CD3+, CD8+, CD20+ and CD68+ positive cells significantly correlated with the mRNA of target genes (Tab. 2). Mononuclear cell invasion in glomeruli was rare and not significant.

Table 2. Association of interstitial infiltrate with gene expression (Spearman's correlation)

Gene transcript	*RANTES*	*MCP-1*	*HGF*	*BMP7*	*TGF-β1*
CD3+	0.61	0.37	n.s.	-0.43	0.32
CD4+	n.s.	n.s.	n.s.	n.s.	n.s.
CD8+	0.49	0.37	n.s.	-0.53	n.s.
CD20+	0.39	0.34	0.31	-0.49	n.s.
CD68+	0.43	0.42	n.s.	-0.47	n.s.

r > 0.279, p<0.05
r > 0.361, p<0.01
r > 0.451, p<0.001
n.s.: not significant

Longitudinal study - univariate analysis

Patients in the "progressor" group were older, had poorer GFR and higher serum creatinine at the biopsy. Morphological evaluation found higher chronic vascular changes in the "progressor" group ($p<0.02$, RR=3.7). Similarly, the higher intrarenal expression of TGF-β1 mRNA and CD68+ cells infiltrate were found in the "progressor" group. There were no differences in the histological presence of crescents, glomerulosclerosis, interstitial fibrosis, ACEi/ARB treatment or used immunosuppression.

Longitudinal study - ROC analysis

A higher TGF-β1, HGF and RANTES intrarenal mRNA expression and higher macrophages infiltration were associated with the risk for disease progression (RR 7.1 for TGF-β1, 5.5 for HGF, 4.9 for RANTES and 7.5 for CD68 respectively) ($p < 0.05$) (Fig. 1). Both univariate and ROC analyses found the interstitial infiltrate to have no significant relationship with the disease progression.

Longitudinal study - multivariate analysis

The influence of multiple variables on IgAN progression from univariate analysis was analyzed by multivariate regression model. CD68+ cells were excluded from this model because of missing data from 3 progressor patients. This model showed advanced chronic vasculopathy (OR=7.1) and higher TGF-β1 expression (OR=12.2) to be associated with two-year disease progression (Tab.4).

Association of TGF-β1 with the chronic vasculopathy

TGF-β1 immunohistochemical staining revealed both the focal positivity within the inflammatory cells rich interstitium and within the thickened smooth muscle artery intima in patients in whom IgAN progressed.

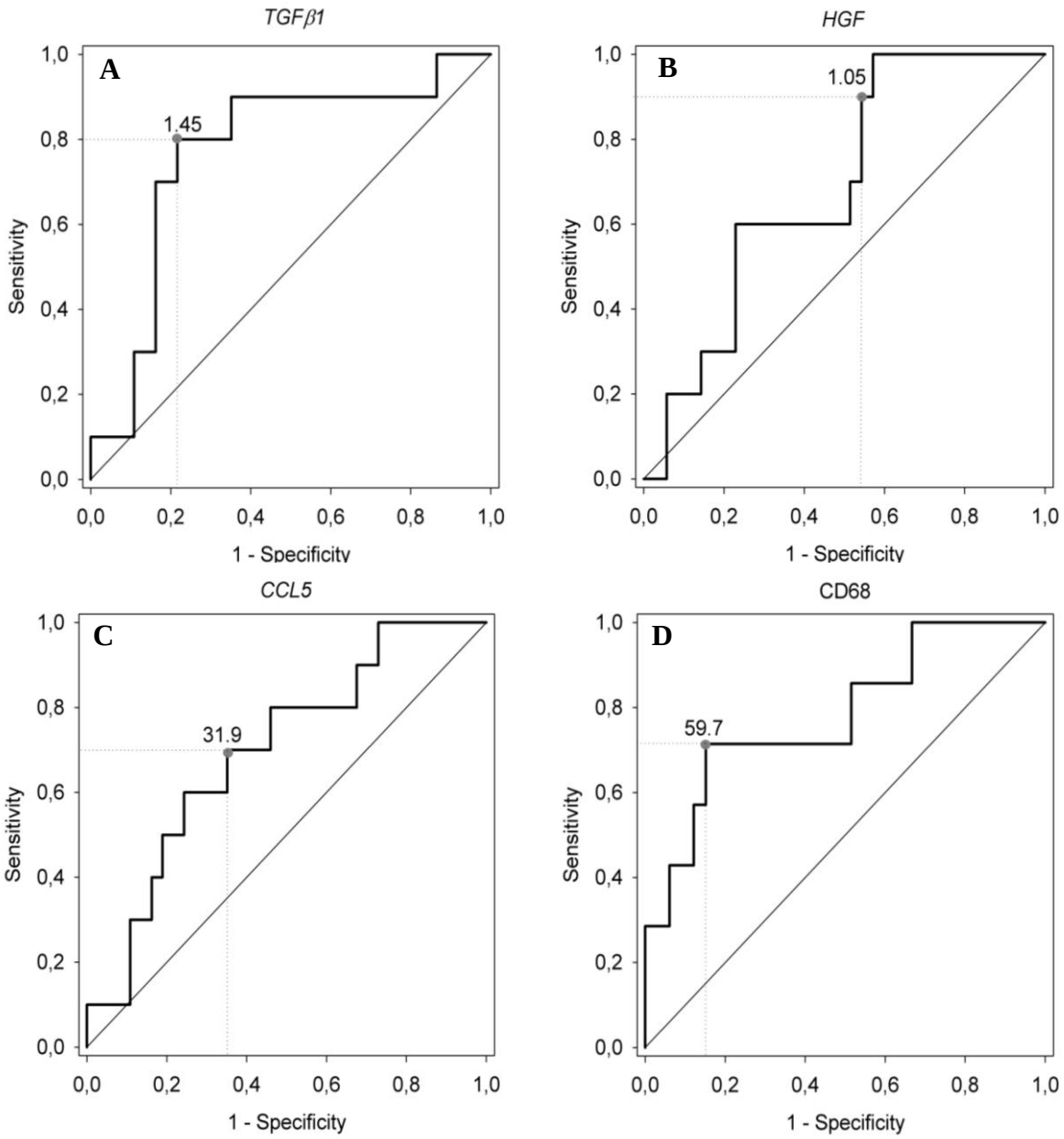

Fig.1. ROC curves for IgAN progression according to the TGF-β1, HGF, CCL5 (RANTES) gene expression and CD68+ cell infiltration. Up-regulation of these markers significantly determined the progression of IgAN at 2 years. *A) TGF-β1 (cut – point = 1.45, sensitivity = 80.0%, specificity = 78.4 %, AUC= 0.77, SE = 0.09) B) HGF (cut – point = 1.05, sensitivity = 90.0%, specificity = 45.7 %, AUC= 0.69, SE = 0.08) C) CCL5(RANTES) (cut – point = 31.9, sensitivity = 70.0%, specificity = 64.9 %, AUC= 0.70, SE = 0.09) D) CD68 (cut – point = 59.7, sensitivity = 71.4%, specificity = 84.8 %, AUC= 0.78, SE = 0.11)*

Table 4. Markers of IgAN progression in multivariate analysis

		Multivariate logistic regression			
	univariate p	**coefficient**	**OR**	**95%C.I.**	**p**
Age (yr)	0.05	-	-	-	n.s.
Creatinine (μmol/L)	0.02	-	-	-	n.s.
GFR (mL/s)	0.00	-	-	-	n.s.
immunosuppression (%)	0.19	-	-	-	n.s.
RANTES >31.9	0.06	-	-	-	n.s.
HGF >1.05	0.07	-	-	-	n.s.
TGF-β1 >1.45	0.01	2.5	12.2	1.6 – 91.0	0.007
cv ≥2	0.02	2.0	7.1	1.0 – 50.2	0.03

n.s.: p>0.10

Progression of renal dysfunction

Intrarenal gene expression in CAN

Patients with biopsy-proven CAN exhibited significantly higher expression of all measured genes compared to the control group. The mRNA expression of *TNF-α* and *RANTES* correlated with the time post-transplant ($P<0.05$). The mRNA expression levels of almost all followed genes correlated with proteinuria: *IL-6* (P<0.001), *IL-10* (P<0.01), *TNF-α* and *MCP-1* (P<0.05). There was a trend towards higher expression of *TGF-β1* and *RANTES* in patients with higher proteinuria.

From histomorphological parameters we found correlation between allograft tubulitis and intrarenal expression of *TGF-β1*, *RANTES*

(P<0.01) and *MCP-1* (P<0.05). We found increased intrarenal expression of *TNF-*α (P<0.05) in biopsies with positive C4d complement component staining (the marker of humoral rejection).

Gene expression in CAN and renal function in the long-term follow-up

Renal function 42 months after the biopsy was evaluated by GFR. ROC curve analysis in controls revealed that *TNF-*α mRNA expression over 0.035 was associated with deteriorated renal function (GFR<0.8 ml/s) at 42 months after the initial biopsy (100% sensitivity, 60% specificity, AUC 0.78). Enhanced *MCP-1* gene expression in the initial biopsy implied an increased risk for renal graft failure in CAN patients within 42 months (OR 5.1; P=0.017). Patients with CAN and enhanced intrarenal expression of *TGF-*β*1* and *MCP-1* at the time of biopsy had significantly shorter graft survival than patients with the low expression of these genes (Figure 6).

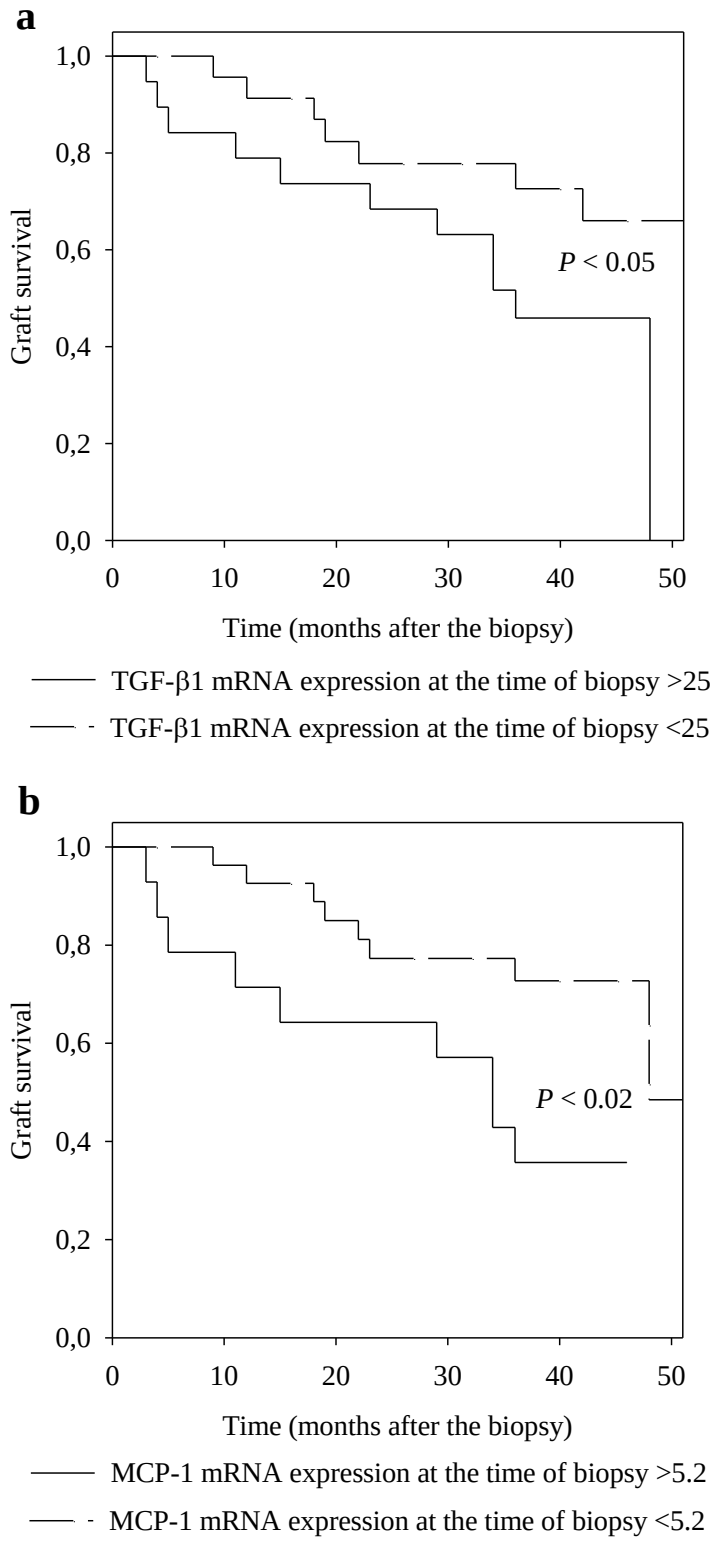

Figure 6: Kaplan-Meier analysis of graft survival in CAN patients. *(a) the influence of TGF-β1 expression (P=0,04). (b) the influence of MCP-1 expression (P=0,018).*

Genes polymorphisms and the kidney graft outcome

All alleles at individual loci were in Hardy-Weinberg equilibrium and the genotype frequencies in the control groups for all polymorphisms were in concordance with the reference HapMap database [27]. There was significant linkage disequilibrium between the *TGF-β1* +869T/C and *TGF-β1* +915G/C loci, with the 69% of the inferred haplotypes consisting of either T-G or C-C.

Neither univariate analysis nor multivariate analysis showed significant difference in the distribution of the genotype frequencies between patients with and without acute rejection, and between patients with CAN or subclinical rejection and individuals with normal 12-months protocol biopsy. No influence of any polymorphism on the graft survival was observed. Haplotype *TGF-β1* [+869G; +915C] seemed to be associated with the presence of subclinical rejection (OR 3.45, 95%CI 1.19-9.99, P=0.023), but the association was non-significant due to the insufficient power.

DISCUSSION

IgA nephropathy (Mb. Berger, IgAN)

The different outcome of IgAN with similar clinical and morphological manifestations suggests its molecular heterogeneity. In this prospective study, we hypothesized that the expression patterns of several genes with the known function in fibrosis regulation along with

immune cells infiltration areassociated with the disease progression. In IgAN diagnosis, we found both. Several gene transcripts and immune cells infiltrate to correlate with the known risk factors of disease progression such as poor renal function and proteinuria. However, in the 24-month follow-up, the IgAN progression was associated only with the chronic vasculopathy and *TGF-β1* gene expression.

Chronic vasculopathy, the consequence of hypertension and aging, causes renal ischemia. As a result of renal ischemia, angiotensin II dependent TGF-β1 overexpression was identified to be a critical component of renal fibrogenesis [28]. TGF-β1 is a central stimulus of the events leading to chronic progressive kidney disease, having been implicated in the regulation of cell proliferation, hypertrophy, apoptosis and fibrogenesis. By multiple mechanisms, this growth factor acts as a major regulator of extracellular matrix production and degradation; it stimulates synthesis of extracellular matrix, enhances expression of integrins, and reduces activities of matrix-degrading proteases [29]. Recently, novel mRNA and proteins signatures related also with TGF-β1 signaling were shown to be predictive for CKD progression in both mice and humans [30]. This observation is in line with the results of our study.

In the cross-sectional part of the study, we showed interstitial infiltrate of T and B lymphocytes along with macrophages to correlate with the up-regulation of chemokines genes that attract lymphocyte and monocyte into the site of injury. These cells are thought to actively participate in fibrogenesis [29]. Proteinuria is widely accepted as a risk

for CKD progression [31], and we showed a clear association of the proteinuria with interstitial infiltrate. Contrary to this cross-sectional data, only macrophage interstitial infiltration but not lymphocyte infiltrate was shown to predict the IgAN progression in the longitudinal part of the study. This contrasts with retrospective studies where tubulointerstitial inflammation determined IgAN prognosis [10, 32]. These studies provided, however, no relevant information regarding the used immunosuppression while in our study, both "progressor" and "non-progressor" groups did not statistically differ in immunosuppressive therapy. This may reflect the presence of active disease phenotype with dense mononuclear infiltrates at the early stage of the disease that was successfully converted.

Progression of renal dysfunction

Intrarenal gene expression

We found all studied cytokines and chemokines to be up-regulated in biopsies revealing CAN. It has been also shown that in acute rejected grafts TGF-β1, MCP-1, RANTES, MIP-1β and MIP-1α expression rise with a higher degree of tubulitis [33, 34]. We found a similar relation of tubulitis with the intrarenal expression of TGF-β1, MCP-1 and RANTES in CAN. This indicates different immunological activity in grafts with diagnosis of CAN, which is reflected also by different intrarenal expression levels of cytokines and chemokines. We can infer that the

risk of graft failure is higher for grafts with active immunological processes than for grafts with immunologically stable disease.

Glomerular proteinuria is a risk factor for the progression of chronic renal failure and interstitial fibrosis [35-38]. Growth factors and cytokines have been shown to be translocated into proximal tubular fluid and activate tubular cells that respond with increased extracellular matrix production and chemokines secretion. It seems reasonable to hypothesize that proteinuria and growth factors ultrafiltration could cause the up-regulation of inflammatory cytokines and growth factors leading to interstitial fibrosis of the transplanted kidney. The intrarenal expression of all studied genes in CAN correlated with proteinuria. The up-regulation of MCP-1 and TGF-β1 heightened the risk for renal graft failure within 42 months and shortened the graft survival time. Based on the above-mentioned results we can recommend a tight monitoring of patients after kidney transplantation with up-regulated intrarenal expression of pro-inflammatory genes. Proteinuria is also an important modifying factor which must require therapeutic intervention.

Possible utilization of gene expression analysis thus resides in identifying grafts with enhanced immunological activity which are at higher risk of graft function deterioration and failure.

Gene polymorhisms

Analysis of cytokine and chemokine gene polymorphisms could help to find kidney graft recipients predisposed to higher cytokine or chemokine gene expression and thus predisposed to higher risk of graft dysfunction.

Recently, the role of these polymorphisms in the susceptibility to the allograft nephropathy in humans has been studied and several groups reported an association with acute and chronic kidney graft rejection. However, these results are often questionable and in some cases also dissimilar [39-42]. Additionally, the results of studies that analysed the influence of cytokine and chemokine gene polymorphisms on the kidney allograft survival are different [26, 43, 44]. There are several potential explanations for the discrepancy between results. These explanations refer to the currently accepted prerequisites for the design of genotype/phenotype association studies [45, 46], to which the previous studies did not closely adhere.

In our study, drawn up to fulfil demands of the sufficient power of tests, no association of *TNF-α*-308G/A, *MCP-1*-2518A/G, *RANTES*-403G/A, -109T/C, -28C/G, CCR2+190G/A, *IFN-γ*+874A/T, *TGF-β1*-869T/C, +915G/C and *CCR5*Δ32 polymorphisms with neither acute rejection or subclinical rejection nor CAN was found.

CONCLUSIONS

In this submitted dissertation, the results of studies performed at the Transplant Laboratory and Department of Nephrology, Institute for Clinical and Experimental Medicine dealing with the influence of some molecular-genetic factors on the kidney transplantation outcome and pathogenesis of glomerulopathies are summarized. The studies were supported by the Internal Grant Agency of the Ministry of Health, Czech

Republic and by the Institute for Clinical and Experimental Medicine. All studies were designed to detect of reliable markers indicated active imunological proces in native kidney disease or during allograft rejection and thereby contribute to more effective patient treatment. We found that:

1. besides known risk factors for CKD progression such as higher proteinuria and poor renal function, in our study we have showed advanced vasculopathy and molecular signatures of fibrogenesis to be associated with the short-term IgAN progression. For adapting treatment decisions to IgAN molecular phenotype in the light of our limited sample size, larger prospective multi-center and molecular marker-based clinical trials are warranted to validate these results.

2. the kidney grafts that suffer from CAN according to the Banff 97 classification differ in the proinflammatory cytokine and chemokine gene expression which influence long-term graft survival. Therefore, these expression patterns rather than the degree of fibrosis may serve as surrogate markers discriminating grafts as the risk for failure.

3. we did not confirm an association of TNF-α-308G/A, MCP-1 -2518 A/G, RANTES-403G/A, -109T/C, -28C/G, CCR2+190G/A, IFN-γ +874A/T, TGF-β1 -869T/C, +915G/C and CCR5Δ32 polymorphisms with acute rejection, subclinical rejection or CAN in our cohort.

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PUBLICATION ACTIVITY:

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