Clinical Nephrology

B1 Primary glomerular diseases: clinical

M184 ANALYSIS OF CYTOKINE-GENE EXPRESSION PROFILES IN CHILDHOOD MINIMAL CHANGE NEPHROTIC SYNDROME USING cDNA ARRAY

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Minimal change nephrotic syndrome (MCNS) is associated with abnormalities of the immune system, including an imbalance of cytokines. Although several studies were carried out to characterize the cytokine profiles in MCNS, consistent profiles of cytokines that might be involved in the mechanism of proteinuria in these patients have not been fully defined. Recently, cDNA array techniques have become available that allow characterization of the mRNA expression status of a large number of genes. To elucidate the comprehensive cytokine-profile in patients with MCNS, the Atlas Cytokine/Receptor cDNA Expression Array which carry 268 cDNA probes of defined human genes (CLONTECH) was employed in this study. Total RNA was extracted from peripheral blood mononuclear cells (PBMC) of five pediatric MCNS patients both in onset and remission stages (steroid-free). After DNAase treatment, cDNA was reverse transcribed from mRNA with random primers incorporating a ^{32}P isotope and applied to a membrane array. After hybridization with a cDNA array, the blots were analyzed with a bio-imaging analyzer, and up/down-regulation was considered to have occurred if there was more or less than doubling or half of the expression level. Selected genes were further confirmed by a conventional RT-PCR analysis; basically using specific primers for genes blotted on the membrane. When compared the gene expression profiles from PBMC of MCNS patients in onset with those in remission, 50 genes were found to be differentially expressed (16 genes were up-regulated and 34 genes were down-regulated in onset compared to remission stage). The up-regulation of IL-13 gene and no up/down-regulation of IL-4 and INF-g genes were consistent with the previous report. In addition, the present study revealed a number of genes whose mRNA expression was not previously known to be up/down-regulated in MCNS. In conclusion, there are several technical issues and limitations in cDNA array techniques, evaluation of mRNA expression profiles by cDNA array analysis may serve as a useful approach to understand the pathogenesis of proteinuria in patients with MCNS.

M185 EOSINOPHIL CATIONIC PROTEIN (ECP) AND SKIN PRICK TESTS IN CHILDREN WITH STEROID SENSITIVE NEPHROTIC SYNDROME (SSNS)

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Childhood minimal change nephrotic syndrome (MCNS) is often associated with allergic symptoms. The association between atopy and nephrotic syndrome may have a causal or non-causal basis. To assess the atopic state of patients with SSNS, serum ECP levels were measured by chemiluminescent enzyme immunometric assay and skin prick tests were done in 32 children with SSNS and 10 age-and sex-matched healthy children without evidence of atopy. Out of the nephrotic patients, 19 children had active disease (Group I) and 13 were in remission (Group II). Among group I, 7 children were frequent relapsers (FR) while 12 were infrequent relapsers (IR) or non-relapsers (NR). We found that 37.5% of our patients had positive skin prick tests. Serum ECP levels were elevated in group I patients [median=25.3 & Interquartile range (IQR)= 13.8-33.6 ng/ml] and group II patients [median= 14.2 & IQR= 12.0-20.2 ng/ml] compared to controls [median= 9.1 & IQR= 7.2-13.5 ng/ml, $p<0.0001$ & 0.006 respectively]. Similarly, patients with negative skin prick tests in group I and group II had higher ECP levels compared to controls (P= 0.007 & 0.07 respectively). Among group I, ECP levels were higher in patients with positive skin prick tests compared to those with negative tests ($p< 0.0001$) and in FR compared to IR and NR (p= 0.05). Moreover, there was an association between the development of frequent relapses and the positivity of skin prick tests (Fisher's Exact= 0.07, relative risk= 6.4 & confidence interval= 1.0-41.2). In conclusion, serum ECP levels are elevated in children with active SSNS. ECP could be considered as one of the neutralizing cations involved in the pathogenesis of proteinuria in these patients. Atopy could be assumed as a risk factor for the development of frequent relapses, so the value of a course of non-steroidal anti-inflammatory drug (as ketotifen) in frequently relapsing nephrotic children should be evaluated.

M186 THE ASSESSMENT OF MORPHOLOGICAL PARAMETERS AND FUNCTION OF PLATELETS IN CHILDREN WITH NEPHROTIC SYNDROME

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Pathogenesis of thromboembolic complications of the nephrotic syndrome has a multifactorial genesis and has not been well explained yes. Up to in spite of hyperfibrynogenemia and decreased concentration of antythrombin III, a big role is attributed to platelets. The aim of the study was the assessment of morphological parameters of platelets and the concentration of platelets derived growth factor, released during the platelets activation in the children with the nephrotic syndrome.

Material: The examinations were carried out in 2 groups of children: I-33 (M-16, F-17) aged 6.8±2.1 with the steroid-sensitive nephrotic syndrome, in whom the examination was carried out twice: A – before the treatment (proteinuria > 50 mg/kg b.w./24 hours, the concentration of albumin <2.5 g%), B – after 6 weeks of prednisone treatment in a dose of 2 mg/kg m.c./24h (without proteinuria, the concentration of albumin > 3.5 g%), II - 34 healthy children (M-18, F-16).

Methods: The platelets count (PLT), mean platelet volume (MPV), plateletcrit (PCT) and platelet dimensional width (PDW) was assessed on MAXEL analyser f. Coulter. The concentration of platelet derived growth factor (PDGF) was measured by ELISA, using monoclonal antibodies f. R&D Quantikine.

Results: The results showed, that in the examined group, before tratment (IA) the amount of platelets was significantly higher than in the control group ($p<0.001$). After the prednisolone treatment decreased amount of platelets was observed, however the values still have exceeded the values in the control group ($p<0.05$). Together with the increase of platelet count, decrease in their volume (MPV) was noticed. The PLT count was inversely correlated with the MPV in the patients group before the treatment (r= -0460, $p<0.001$). Plateletcrit (PCT) and platelet dimensional width (PDW) both before and after the treatment did not differ from the results of the control group ($p>0.05$). The concentration of platelet-derived growth factor (PDGF) in the group of children with nephrotic syndrome, before the treatment (A) was 5,76±2,98 ng/ml, and decreased to 3.39±1.76 ng/ml after the treatment (B). Although in both examinations the concentration was higher than in healthy controls ($p<0.05$). The positive linear correlation between PLT and PDGF (r= 0.419, $p<0.05$), and negative linear correlation between the MPV and PDGF (r= - 0,459, $p< 0,05$) in patients group children in examination A was found. The particular analysis of the results showed that, the disturbances in morphology and function of platelets were higher in the children with exacerbation of nephrotic syndrome (albumin <2g%), than in the group in remission (albumin 2-2.5 g%).

Conclusion: In the children with the nephrotic syndrome simultaneously to exacerbation of disease increased platelets count (PLT), decrease in their volume (MPV) and increase of their activity, expressed in higher concentration of platelet derived growth factor (PDGF) were noticed.

M187 ASSOCIATION ANALYSES OF GLUCOCORTICOID RECEPTOR GENE POLYMORPHISMS WITH STEROID-RESISTANT IN IDIOPATHIC NEPHROTIC SYNDROME OF CHILDREN

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Idiopathic nephrotic syndrome (INS) children are mainly treated with glucocorticoids. The majority of patients are steroid-sensitive (SSINS), while steroid-resistant occurs in a subset of INS children (SRINS). To date, the mechanisms underlying steroid-resistant remain unknown. Since the biologic effects of glucocorticoids are mediated by the glucocorticoid receptor (GR), it can be hypothesized that some variations in GR play an important role in SRINS. The study aimed to screen the GR gene (NR3C1) for polymorphisms in genomic DNA samples from SSINS, SRINS children and control group, and determine the polymorphisms in the NR3C1 gene and their association with steroid-resistant INS of children.

Genomic DNA was isolated by standard techniques, from 64 control samples, 39 steroid-resistant, and 67 steroid-sensitive INS children, respectively. All the NR3C1-coding exons and some intron-exon boundaries were amplified by polymerase chain reaction (PCR). -For polymorphism screen, PCR products were analyzed by denaturing high-pressure liquid chromatography (DHPLC). DNA fragments with aberrant elution profiles were re-amplified and sequenced directly.

Ten aberrant elution profiles of DHPLC were identified in SRINS, SSINS and controls. Among them, 6 novel polymorphisms and 6 previously reported polymorphisms were confirmed by sequencing (198G>A, 200G>A, IVSD-16G>T, 1896C>T, 2166C>T, 2430T>C; novel, 1206C>T, 1374A>G, IVSG-68_IVSG-64delAAAAAA, 2193T>G, IVSH-9C>G, 2382C>T); and 3 groups of polymorphisms were in complete linkage disequilibrium, resulting in 3 different haplotypes ([198G>A + 200G>A], [1374A>G + IVSG-68_IVSG-64delAAAAAA + IVSH-9C>G + 2382C>T], [1896C>T + 2166C>T + 2430T>C]). The last two genotypes were first reported. The genotype frequencies of the 2 newly found haplotypes were 10.3% vs 1.5% in SRINS and SSINS, and 15.4% vs 7.5% in SRINS and SSINS, respectively. Data analysis showed that the two haplotypes confers strong risk of steroid-resistance (7.54-fold and 2.25-fold respectively). Other polymorphisms were relatively rare detectable both in patients and controls.

We had characterized 6 novel polymorphisms in the NR3C1 gene, and found significant evidence for two groups of complete linked haplotypes located at the NR3C1 gene. -The results of our studies indicated that the two newly found haplotypes were associated with steroid-resistant idiopathic nephrotic syndrome of children, which might be responsible for steroid-resistant in partial idiopathic nephrotic syndrome of children.

M188 IL4, IL 8, IL 10 AND IL 18 m-RNA EXPRESSION IN PERIPHERAL BLOOD MONONUCLEAR CELLS ARE UP-REGULATED IN ACTIVE PHASE MINIMAL CHANGE NEPHROTIC SYNDROME PATIENTS

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It has been assumed that glomerular permeability factors produced by lymphocyte are responsible for minimal change nephrotic syndrome (MCNS). However, the nature and the regulatory mechanisms of these factors are poorly understood. We hypothesize that cytokines produced by peripheral blood mononuclear cells (PBMC) may modulate the production of these glomerular permeability factors. Therefore we evaluated and compared the cytokine mRNA expression in PBMC from healthy volunteers and active phase MCNS patients using quantitative real time PCR method. Mononuclear cells were obtained from peripheral blood (N: MCNS patient = 6, healthy volunteer = 6) using density gradient centrifugation method. Total RNA was extracted, reverse transcribed and amplified using specific primers.

Expression of IL-4, IL-8 and IL18 m-RNA in PBMC from MCNS patients were increased by 9.3-fold, 11.4-fold, and 1.7-fold, respectively, as compared to healthy volunteers. IL-10 mRNA was detected in MCNS patients but not detectable in healthy volunteers. No significant difference in expression of β-actin, IL-1β, IL-6, interferon-γ, proliferating cell nuclear antigen or monocyte chemotactic protein-1 m-RNA was demonstrated. We hypothesize that these up-regulation of specific cytokine mRNA expression may contribute to the pathophysiology of MCNS.

M189 COMPREHENSIVE GENE EXPRESSION PROFILING ANALYSIS IN MINIMAL CHANGE NEPHROTIC SYNDROME (MCNS) USING A cDNA ARRAY

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Derangement of the immune system, especially in T lymphocytes, has been considered to play a crucial role in the pathogenesis of minimal change nephrotic syndrome (MCNS). Although some permeability factors in circulation, such as a lymphokine, have been postulated, the actual pathogenic factor still remains unknown. To elucidate the mechanism of proteinuria in MCNS, we performed a comprehensive gene expression profiling analysis using a cDNA array in both remission and relapse stages. Total RNA was purified from peripheral blood mononuclear cells of five patients with MCNS, both in remission and relapse stages. Two patients with nephorotic syndrome caused by membranous nephropathy (MN) and one healthy subject were analyzed as controls. After DNase treatment, randomly labeled cDNA probes were prepared by reverse transcription using specific primers for each arrayed gene and hybridized to the cDNA array. A total of 1,176 arrayed genes were quantitatively evaluated using a bio-imaging analyzer. To eliminate individual biases, we compared the expression change between remission and relapse stages in each patient. We screened for genes of which the expression ratio differs more than 1.5 times in more than three out of five cases. Expression profiles of mononuclear cells resembled one another regardless of whether in remission or relapse stages, however, they were widely different from those of mesangial cells. Almost all of the ten most strongly expressed genes were common in all samples and were in good agreement with known expression in lymphocytes or monocytes/macrophages. General expression patterns of MCNS in relapse stages were closer to MCNS in remission stages than to those of MN, suggesting characteristic expression profiles in MCNS, irrespective of nephrotic status, were revealed. Concerning previously reported permeability factors, such as IL-2, IL-8, TNF-alpha, VEGF and their related genes, the expression changes were observed in less than 3 cases, except for TNFRSF7. We screened for 25 candidate genes which may relate to massive proteinuria. We are further screening for genes by quantitative RT-PCR to confirm the expression changes of each candidate genes.

In conclusion, comprehensive gene expression profiling using a cDNA array in MCNS may serve as a significant approach for elucidating its pathogenesis.

M190 DOES PLASMA LEPTIN LEVEL CHANGE DURING THE TREATMENT WITH CORTICOSTEROIDS IN THE PATIENTS WITH NEPHROTIC SYNDROME?

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Leptin (Le) is a newly discovered hormone, which has a close relation with fat metabolism and plays an important role in the nutrition. Plasma concentrations of Le can be down-regulated by its loss into the urine and also up-regulated by liver synthesis in the patients with nephrotic syndrome (NS). Inzulin and corticosteroids (CS) increase plasma levels of Le. The aim of the study was to investigate the plasma levels of Le, soluble receptor for Le (sLe-R) and other parameters before and after the treatment with CS in the group of patients with NS and their relationship with albuminemia and proteinuria.

The study group consisted of 15 men and 15 women (mean age 49 ± 13.7 years) with newly diagnosed NS verified by renal biopsy. The mean serum creatinine level was 128.5 ± 34.8 umol/l. The diagnoses leading to NS were minimal change nephropathy in 13 cases, focal segmental glomerulosclerosis in 11 cases and membranous nephropathy in 6 cases. The following parameters were investigated before the CS treatment (period 1) and further 1 month (period 2) and 6 months (period 3) after the start of the CS treatment: plasma Le, sLe-R, insulin-like growth factor-1 (IGF-1); serum albumin, C-peptide, glycosylated hemoglobin, cholesterol, triglyceride, cholinesterase, creatinine. Body mass index, hydratation status, proteinuria, area under curve of glucose (AUC-G) and insulin (AUC-I) were also investigated or calculated.
Plasma levels of Le in the patient group were 13.1 ± 7.6 ng/ml initially and did not change significantly during the CS treatment, but were significantly increased in period 2 when compared with healthy controls (15.8 ± 10.3 vs. 7.36 ± 4.2 ng/ml, $p < 0.05$). We did not find significant difference between men and women in plasma Le and no correlation was found between plasma Le and other investigated parameters. Significant negative correlation was found between sLe-R and IGF-1 in period 1 ($r = -0.45$, $p < 0.05$) and positive correlation between IGF-1 and C-peptide also in period 1 ($r = 0.47$, $p < 0.05$).
We did not find meaningful changes in plasma Le levels in the patients with NS during CS treatment. The results of our relatively unique study on Le dealing with long-term follow-up of patients with NS suggest, that regardless prominent metabolic alterations present in NS the plasma levels of Le and sLe-R remain relatively stable, and that the regulation of Le in this setting is probably complex and multifactorial.
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M191 A STUDY OF TUBULAR DYSFUNCTION IN IDIOPATHIC NEPHROTIC SYNDROME

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Nephrotic Syndrome is considered to be a common manifestation of Glomerular diseases, and tubular function defects have not been given much importance in Nephrotic Syndrome. In this study we compared the tubular function in 30 (23 M 7 F)patients of Idiopathic Nephrotic Syndrome with the age range between 9 years to 50 years with those of controls (6 M,4 F)with the age range between 10 years to 45 years.All the patients were off steroids&diuretics at the time of study. Glycosuria was present in 5 patients (16.66%) despite of having normal blood glucose levels while none of the controls had Glycosuria On analysis by paper chromatography we found that urine of our controls contained small amounts of amino acids lysine,cystine,cysteine,glutamine,taurine,glycine alanine&threonine but in each of our patients the output of these aminoacidswas considerably increased&most of our patients contained additional aminoacids-tyrosine,valine,methionine,leucine,isoleucine&phenylalanine.
Fractional excretion of Sodium,Potassium,Calcium&Inorganic phosphorous were Found to be higher in Nephrotic Syndromeas compared to controls. It was observed that Patients with marked Proteinuria had more significant tubular dysfunction.

M192 ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH NEPHROTIC SYNDROME

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Nephrotic syndrome (NS) is associated with dyslipoproteinemia and increased cardiovascular risk. An early phase of atherogenesis is endothelial dysfunction (ED). The purpose of our study was to investigate any possible association between NS and ED.
We included in our study 14 patients admitted to our department with the diagnosis of NS (group A) and 14 controls (group B), matched for age, sex, weight. We assessed in both groups the endothelial function by measuring the post ischemic flow mediated dilatation (PIFMD) of the brachial artery using ultrasonography. We measured in both groups the plasma levels of urea, creatinine, albumin, glucose, total cholesterol, HDL, LDL, triglycerides, fibrinogen, and CRP.
PIFMD in group A, was statistically lower than group B (4.5±0.6% vs. 7.1±0.5%, $p<0.01$).
The plasma levels of cholesterol (390±73 mg/dl group A vs. 171±68 mg/dl group B), LDL (254±46 vs. 103.2±29 mg/dl) and triglycerides (228±33 vs. 81.5±28) were significant higher in group A.
There were no significant differences in urea, creatinine and CRP levels but fibrinogen was significantly higher in group A than in group B (510 ± 3 mg/dl vs. 244 ± 61 mg/dl – $p<0.01$). Multivariate regression analysis showed that LDL ($P<0.05$) and albumin plasma levels ($P<0.01$) were strong independent negative predictors for PIFMD.Our data suggest that patients with NS have compromised endothelial function. It seems from our study that dyslipidemia and hypoalbuminemia has an important role to this and consequently to increased cardiovascular risk.

M193 ELEVATED URINARY NAG, TGFβ AND MCP-1 EXCRETIONS DURING IMMUNOSUPPRESSIVE TREATMENT OF PATIENTS WITH NEPHROTIC SYNDROME ARE PREDICTORS OF POOR THERAPEUTIC RESPONSE

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The aim of this study was to evaluate the changes in urinary NAG, TGFβ and MCP-1 excretions (which are activity/progression markers of the disease) in patients with nephrotic syndrome (NS). Twenty four patients with NS and biopsy proven primary glomerulonephritis (GN) (16 patients with MesPGN, 5 with FSGS and 3 patients with membranous GN) were studied. TGFβ and MCP-1 were measured in urine by ELISA method whereas NAG by colorimetric method before and after six and twelve months of immunosuppressive (IMS) therapy. Urinary excretions of these cytokines and NAG in 10 healthy subjects served as control.
At the beginning of the study urinary TGFβ (382.9±319.1 ng/gCr), MCP-1 (778.6±465.3 ng/gCr), and NAG excretions (19.4±15.2 U/gCr) were significantly higher in all nephrotic patients as compared to healthy subjects (171.9±30.8 ng/gCr, 178.0±80.0 ng/gCr, 4.1±2.0 U/gCr, respectively).
All patients were treated with corticosteroids and cyclophosphamide or mycophenolate mofetil. After 6 and 12 months of IMS therapy a negative correlation between TGFβ, NAG and creatinine clearance ($p<0.005$), and a positive correlation between NAG and TGFβ ($p<0.05$), and NAG and MCP-1 ($p < 0.05$) were found. In patients with the remission of NS due to the treatment (reduction of proteinuria from 6.8± 4.4g/24h to 0.2± 0.1 g/24h - responders) a significant decrease of urinary NAG activity was observed (from 22.7±14.6 to 5.7±4.7 U/gCr) . In patients with persistent proteinuria urinary NAG (14.0± 9.3 U/gCr), TGFβ (497.4 ± 303.2 ng/gCr and MCP1 (588.9± 277,3 ng/gCr) excretions were significantly higher as compared to responders (TGFβ 226,8± 110.8 ng/gCr, $p<0.008$; MCP-1 392.9±370 ng/gCr, $p= 0.004$; NAG 5.5±4.7 U/gCr, $p = 0.005$, respectively) and to healthy subjects. The results showed that unchanged urinary excretions of NAG, TGFβ and MCP1 are predictors of poor response observed during IMS treatment in patient with primary GN and NS.

M194 PRIMARY NEPHROTIC SYNDROME IN CHILDREN: WHAT ARE THE PREDICTORS?

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To study the clinical course and identify factors at presentation that predict the development of renal failure in children with nephrotic syndrome, a retrospective analysis was done on 108 patients who were diagnosed as nephrotic syndrome from 1981-2201. The predicators of renal outcome include age, gender, systolic and diastolic blood pressure, serum creatinine, presence of hematuria, severity of proteiuria, urinalysis, kidney sonography (all at time of presentation) and type of renal pathology. Thirty –three

patients (30.6%)developed renal failure. Present of hypertension and low urine specific gravity at time of diagnosis had most correlation for developing renal failure (P<0.05). No other factor showed significant association with renal failure.Conclusion: For a patient with primary nephrotic syndrome, presence of hypertension and low urine specific gravity at time of presentation might be used to predict the risk of developing renal failure.

M195 ADOLESCENT ONSET NEPHROTIC SYNDROME IN INDIA: CLINICAL FEATURES AND HISTOPATHOLOGICAL SPECTRUM

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The adolescent population signifies the transitory period where the frequency of occurrence of different histopathological lesions in patients with nephrotic syndrome is different from that seen in the paediatric population less than 12 years of age as well as that seen in adults. The types of glomerular pathology encountered in adolescent population with nephrotic syndrome have not been well characterised. We evaluated clinical features, laboratory data and histopathology of 163 patients with nephrotic syndrome having its onset between 12 to 18 years of age seen at this Institute between January 1989 and November 2000. The commonest cause of idiopathic nephrotic syndrome was minimal change disease (MCD) in 49 (33.1%) patients followed by focal segmental glomerulosclerosis (FSGS) in 43 (29%), membranoproliferative glomerulonephritis (MPGN) in 26 (17.6%) cases, mesangial proliferative glomerulonephritis in 15 (10.1%), membranous glomerulopathy (MGN) in 6 (4.0%), sclerosing glomerulonephritis, crescentic glomerulonephritis and IgA nephropathy in 3 (2.0%) patients each. Fifteen (9.2%) of these 163 patients had a secondary cause for their nephrotic syndrome. Secondary amyloidosis (53.3%) was the commonest followed by lupus nephritis (33.3%) and diffuse proliferative glomerulonephritis (14.4%). Of the 56 (37.8%) patients with microscopic hematuria, 18 (32.1%) had MPGN, 15 (26.8%) had FSGS and 7 (12.5%) had MCD. Of the 48 (32.4%) patients with hypertension, the commonest lesion was MPGN in 18 (37.5%) cases followed by FSGS in 12 (25%) cases and only 6 (12.5%) patients had MCD. The commonest cause of steroid resistant state was FSGS seen in 13 (35.1%) patients followed by MPGN seen in 8 (21.6%) cases. Among the steroid dependent patients MCD was seen in 57.1% and FSGS in 28.6% of cases. When biopsies done after 1996 were compared with those done before 1996, the incidence of FSGS was seen to have increased from 19.2% to 40.0% (p<0.01).
We conclude that the commonest cause of idiopathic nephrotic syndrome among adolescents is MCD (33.1%) closely followed by FSGS (29%), with 9.2% having a secondary cause. Adolescent nephrotics with microhematuria, hypertension at presentation as well as a steroid resistant state have lesions other than MCD. The incidence of FSGS has increased in the second half of last decade. In view of the high incidence of lesions other than MCD it preferable to biopsy all adolescents with nephrotic syndrome initially rather than treat them empirically with steroids.

M196 ★ EXPRESSION OF GLUCOCORTICOID RECEPTORS IN CELLS OF PERIPHERAL BLOOD IN CHILDREN WITH NEPHROTIC SYNDROME

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Flow cytometry method has been used to determine the expression of glucocorticoid receptor (GCR) in cells of peripheral blood in children with nephrotic syndrome. The aim of work was to assess the expression of GCR in the lymphocytes (CD3/GCR) and the monocytes (CD14/GCR) of peripheral blood of 23 (4.9±2.7 years) children with the steroid-sensitive nephrotic syndrome: A - before treatment, (proteinuria > 50 mg/kg b.w./24 hours, serum albumin <2.5 g%), B – after 4-6 weeks of prednisone treatment, without proteinuria (serum albumin > 3 g%), C – in remission (3 months after withdrawal of proteinuria and without any treatment). The expression of the receptors CD3/GCR and CD14/GCR has been determined by Berki method using Coultier flow cytometry. The results showed in examination A the expression of CD3/GCR was 61,8±18,3% and CD14/GCR was 43.6,8±20,3% and did not differ from the results of normal group (p>0.05). However after treatment (B) the GCR expression in the lymphocytes was 50% (p<0,001) and in the monocytes about 20% lower (p<0.05). In remission (C) the GCR expression increased and did not differ from the results before treatment (p> 0.05). After prednisone (B) treatment the serum cortisol concentration was decreased in comparison to group A and C (p<0.001). The positive correlation between the serum cortisol concentration and the expression of CD3/GCR was found (r=0.504, p=0.02). Decrease of GCR expression in monocytes has not been correlated with cortisol concentration. In conclusion we report that in the children with the steroid-sensitive nephrotic syndrome, prednisone treatment causes the temporary decrease of the GCR receptors expression in the lymphocytes. The positive correlation between the GCR expression and serum cortisol was found.

M197 EFFECTS OF 3 MONTHS FLUVASTATIN TREATMENT ON LIPID METABOLISM AND PROTEIURIA IN PATIENTS WITH NEPHROTIC SYNDROME

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Hyperlipidemia in nephrotic syndrom (NS) results from increased synthesis and decreased catabolism of lipoproteins and is characterized by high total cholesterol (Tchol), triglecerides (TG), LDL and VLDL levels while HDL may be normal. Medical treatment of this disorder is highly controversial. We evaluate the effect of HMG-Co A reductase inhibitor Fluvasatin on lipid disorders and proteinuria in patients with NS. 7 F and 12 M, average age-39 with glomerular filtration rate (GRF) over 90 ml/min and NS with sever hyperlipidemia Tchol > 8,5 mmol/l were investigated. 9 patients were with membranous glomerulonephritis (GN), 4 -with FSGS, 3- with mesangiocapillaris GN and 3- with lupus nephritis. All pts. were treated with corticosteroids alone or in combination with cytotoxic drugs, because of the illness activity. 4-hour creatinin clearance was used to estimate GFR. Fluvastatin was administered 40 mg/d for 3 months. Tchol, TG, LDL, HDL and 24 hours proteinuria were examined before the start of the study and after 3 months. Administration of Fluvastatine led to a significant reduction of lipid fractions:

Tchol mean value (MV)	before the treatment 9,3 3,2	and after- 5,9 1,32	p<0.001
TG: MV	before -5,9 1,87	and after-2,74 1,04	p<0,01
LDL: MV	before -6,351,34	and after- 3,57 0,81	p<0.001
HDL levels were normal in all pts.: MV	before- 1,61 0,53	and after- 1,04 0,23	p=0.01
No significant decrease in proteinuria was observed: MV	before 4,21 2,4	and after-3,03 1,68	p= 0.10

Fluvastatin led to a significant reduction of hyperlipidemia in pts. with NS receiving steroids regardless of the fact that the nephrotic proteinuria remained and was not affected by immunosuppressive treatment. To evaluate the effect of satins on proteinuria a long-term study with carefully selected pts without immunosuppressive therapy is needed. HMG CoA reductase inhibitor Fluvastatine have an important role in complex treatment of NS and proved to be effective and save in correction of hyperlipidemia which is one of the important cardiovascular risk factors.

M198 A NEW THERAPEUTIC APPROACH OF STEROID- AND CYCLOPHOSPHAMIDE- RESISTANT ADULT NEPHROTIC SYNDROME

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The treatment of steroid- and cyclophosphamide (CTX)- resistant adult nephrotic syndrome (NS), caused by glomerular disease, is a big worldwide problem. There are a lots of new therapeutic approaches may be sought in

its treatment, but in some cases, remission cannot be achieved with lots of methods. We successfully made these patients got remission by using continuous infusion of dexamethasone (DEX) through catheters inserted renal artery as an inducing treatment.
There were five patients with NS, aged 21 to 54 years old, 3 males and 2 females, received this treatment. All of them were undergone renal biopsy. One had minor change disease, one had focal segmental glomerulosclerosis and the others had membranoproliferative glomerulonephritis. Although they orally took prednisone of 1mg/kg per day for more than two months; three of them received impulsing therapy of CTX at the same time, they still had heavy proteinuria and edema. Patients were punctured at one side of groin into femoral artery, and inserted two catheters from the femoral artery to each main renal artery. Each patient was given the total dosage of DEX of 7.5mg to 10mg per day. DEX was dissolved in saline solution and equally divided into two syringes, then continuously infused into each renal artery through the catheters by pumping. After seven to ten days, infusion of DEX was stoped and catheters were pulled out. The patients took prednisone of 45mg to 60mg per day again for another 20 to 30 days, and then reduced 10mg of prednisone. After this prednisone was gradually reduced to 5mg about one year and stopped. After one month of the therapy, all the patients got remission, proteinuria from 7.3±2.5g to 0.51±0.33g ($p<0.01$), serum albumin from 21.8±8.6g to 38.2±7.9g ($p<0.05$), and edema almost completely disappeared. All the patients were followed up 8 to 28 month, and had no relapse, their renal function were better than before they were treated.
We conclude that direct infusion of steroid into each renal artery has inducing effects on the treatment of steroid- and CTX- resistant adult patients with NS, implying steroids have some direct effects on kidneys.

M199 THERAPY OF FIRST EPISODE OF STEROID RESPONSIVE NEPHROTIC SYNDROME: A RANDOMISED CONTROLLED TRIAL

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Recent evidence indicates that in children in their first episode of steroid responsive nephrotic syndrome (FESRNS) the risk of relapse is significantly reduced with increased duration and dose of prednisone (P). Moreover, the ratio of total dose to duration against relative risk shows that the reduction in relapse risk is primarily associated with an increase in duration not dose (Cochrane Renal Group,2000). To verify this observation we conducted a randomised controlled trial comparing the benefits and the toxicity of two corticosteroid regimes in preventing relapse in FESRNS: "Dose" regime (D): P 2 mg/kg/day for 6 weeks, followed by alternate-day P at the same dose for 6 weeks, thereafter the alternate day dose is decreased every two weeks by 0.25 mg (total duration of therapy: 26 weeks= 6 months). "Length" regimen (L): a methylprednisolone pulse i.v. (20 mg/kg/day) for three days; from the fourth day P 1 mg/kg/day for 6 weeks, followed by alternate-day P at the same dose for 6 weeks, thereafter the alternate day dose is decreased every two weeks by 0.25 mg (total duration of therapy: 26 weeks= 6 months).Two groups of ten children with FESRNS were treated, respectively, with regime D and L. A third group of ten children with FESRNS, treated with APN standard P regime (S): P 2 mg/kg/day for 4 weeks, followed by alternate-day P at the same dose for 4 weeks, thereafter the alternate day dose is decreased every week by 0.25 mg (total duration of therapy: 12 weeks= 3 months), was considered as control group.The primary outcome measure was considered the prevention of relapse as measured by the number of children with and without relapse at 12 months of the study. No difference in the mean age, sex ratio, values of proteinuria and other clinical and biochemical parameters of NS between the three groups was found. Remission of NS was achieved after a mean time of 4.6, 4.1 and 6.4 days, respectively, in group D,L and S. Three pts (30%) of group D, 7 (70%) of group L and 5 (50%) of group S suffered the first relapse after a mean time of 7.3, 4.1 and 3.0 months, respectively. No significant difference in toxicity between the groups was registered. Our results indicate that the reduction in relapse risk in children with SRNS is associated with an increase in both duration **and** dose of P in the treatment of their first episode.

M200 C_2 LEVELS IN CYCLOSPORIN TREATED PATIENTS WITH NEPHROTIC SYNDROME

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Cyclosporin-A (CsA) is used in the treatment of nephrotic syndrome due to various types of glomerulonephritis (GN). Although it usually induces remission of nephrotic syndrome it is a potentially nephrotoxic drug. Studies in kidney transplanted patients show that blood levels two hours after administration of the drug (C_2) represent more accurately the drug exposure than trough levels (C_0). C_2 levels in CsA treated patients with GN were estimated in this study in relation to the effect of treatment. Forty-two nephrotic patients (M/F 21/21), with well-preserved renal function (mean serum creatinine 1.04± 0.3 mg/dl) were included. The original diagnoses were minimal changes disease (MCD), focal segmental glomerulosclerosis (FSGS), membranous nephropathy (MGN), IgA nephropathy (IgAN) and lupus nephritis (LN). All patients were treated with prednisolone (0.5 mg/kgBW/d initially, followed by gradual tapering) and CsA (2-3 mg/kgBW/d), for 24 months. CsA dose was adjusted according to C_0 levels (target: 90-100ng/ml). C_0 and C_2 blood levels were determined by fluorescence polarization immunoassay (FPIA) at regular intervals.
Remission (complete or partial) and relapse rate of nephrotic syndrome is shown in the table.

Nephrotic syndrome	MCD (n=8)	FSGS (n=4)	MGN (n=18)	IgAN (n=6)	LN (n=6)
Complete remission	8 (100%)	1 (25%)	7 (39%)	3 (50%)	2 (33%)
Partial	–	2 (50%)	7 (9%)	3 (50%)	4 (77%)
No remission	–	1 (25%)	4 (22%)	–	–
Relapse	2 (25%)	–	5/14 (36%)	1 (17%)	–

The mean C_0 CsA levels were 93± 9 ng/ml and the corresponding C_2 levels were 498± 113 ng/ml. Lower or higher to the expected C_2 levels were found in 6 patients (14%). Low C_2 levels (340± 83 ng/ml) were found in 3 patients (original diagnoses FSGS and MGN). One of these had complete and two partial remission of nephrotic syndrome. High C_2 levels (680± 127 ng/ml) were found in 3 patients (original diagnoses MCD, FSGS and LN). Two of these had remission and one persistent nephrotic syndrome.
Deterioration of renal function was observed in 3 patients with MGN (17%), 1 with FSGS (25%) and 1 with IgAN (17%). Two of these patients had persistent nephrotic syndrome and 3 had frequent relapses. No relation of C_0 and C_2 levels with serum creatinine was observed.
In conclusion, small doses of CsA with prednisolone are effective in the treatment of nephrotic syndrome due to various GN. The response of nephrotic syndrome to CsA is related to the type of GN. Although no significant differences in the therapeutic effect of CsA was observed with the use of C_0 or C_2 levels, the latter might be proved useful in determination of the optimum CsA dose.

M201 CONCOMITANT ADMINISTRATION OF CYCLOSPORINE AND KETOCONAZOLE IN STEROID NON-RESPONSIVE NEPHROTIC SYNDROME

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The deliberate use of ketoconazole to reduce the need for cyclosporine is not new, but it is particularly relevant because of the high cost of cyclosporine. Many studies have documented this benefit in renal and cardiac transplants, but this co administration has not reported in nephrotic patients. Other theoretical advantages of ketoconazole co administration include a reduction in the rate of infection because of the drug's broad antimicrobial effects. Also, a decrease in the level of low-density lipoprotein (LDL) cholesterol reduces the level of LDL-bound cyclosporine, leaving a higher level of free cyclosporine. Possible disadvantages include the known hepatotoxicity of ketoconazole (particularly since cyclosporine itself is mildly hepatotoxic) and the possible emergence of resistant strains of fungi and yeast.
This study included 207 nephrotic patients who were steroid non-responsive and received cyclosporine therapy. Among those patients 153

received daily ketoconazole therapy in a dose of 50 mg with concomitant decrease 50% of the cyclosporine dose. The majority of our cases were children (175 were below 15 years) and male to female ratio was 2:1. The great majority of the study populations received the drugs for 1-2 years. Patients who received cyclosporine and ketoconazole were comparable to patients who received cyclosporine alone regarding age, sex, the duration of renal disease, renal pathology the severity of nephrotic syndrome, renal function, hepatic function and steroid response.
Co-administration of ketoconazole improved the response to CsA therapy and decreased the frequency of renal impairment and hypertension. The hepatic function was similar in both groups. The co-administration of ketoconazole significantly reduced mean doses of CsA from 2.9 ± 0.9 to 1.6 ± 0.6 mg/kg/day (45% dose-reduction). Consequently, the cost of treatment, including ketoconazole expenses, was significantly reduced from 248.8 ± 90.6 to 163.1 ± 54.6 Egyptian Pound/patient/month (35% cost-reduction). From this study, we conclude that co-administration of low dose ketoconazole with cyclosporine in steroid non-responsive nephrotic patients is safe. This combination not only reduces the costs dramatically but also improves the response to cyclosporine and decreases its complications.

M202 A SINGLE CENTER REPORT ON LONG-TERM EFFICACY AND SAFETY OF CYCLOSPORINE (CSA) TREATMENT IN 207 PATIENTS WITH IDIOPATHIC NEPHROTIC SYNDROME

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This study reports the results of CsA treatment in 207 patients with INS of whom 175 were children. One hundred and eight patients were steroid-dependent. The underlying pathology was focal segmental glomerulosclerosis (FSGS), minimal change disease (MCD), diffuse mesangial proliferation (DMP), membranous nephropathy (MN) and membranoproliferative glomerulonephritis (MPGN) in 126, 48, 15, 10 and 8 patients respectively. All patients had normal renal function before CsA therapy. CsA induced complete remission in 137 (66.2%) and partial remission in 17 (8.2%). The rest of patients were resistant to therapy. Response to CsA was significantly better in: children compared with adults ($p = 0.04$); steroid dependent patients versus steroid resistant ones ($p = 0.001$); MCD, DMP and FSGS compared with other pathological lesions ($p = 0.003$) and in those who had lower quantities of pre-treatment proteinuria ($p = 0.02$). CsA was received for a period of 22.16 ± 12.21 (6-72) months. Discontinuation of the drug in 37 patients resulted in relapse in 73% while the remaining 27% maintained remission until the last follow up (11.1 ± 5.3 months). Eighteen out of 26 patients showed complete remission when CsA was resumed. Renal dysfunction (serum creatinine) developed in 17 patients (8.2%) of whom 11 recovered completely on drug discontinuation. Hypertension developed in 36 (17.4%) patients while hypertrichosis 108 (52.2%) and gum hyperplasia 51 (24.6%) were the most frequent CsA-related side effects. With the exception of hypertrichosis, all side effects were significantly more prevalent in CsA-resistant patients. We conclude that CsA is generally effective in the treatment of INS. Renal dysfunction and hypertension may be CsA-induced or due to disease progression.

M203 ONCE A DAY CICROSPORIN MICROEMULSION, NEORAL® THERAPY GUIDED BY ABSORPTION PROFILES IS USEFUL, SAFE AND COST-SAVING IN REFRACTORY NEPHROTIC SYNDROME

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Ciclosporin (CSA) is well known to have potent effects on nephrotic syndrome (NS) to introduce remission and/or to prevent the recurrence of NS. A conventional ciclosporin, Sandimmune®, has a clinical problem of the unstable absorption, and both inter-/and intra-individual difference of CSA were considerable. A conventional therapy using CSA for nephrotic patients is a twice a day administration under control of the trough blood level. No clinical data had supported the efficacy, safety and cost-saving benefit of this kind of CSA treatment. In order to achieve more effective administration and avoid the long-term nephrotoxicity of CSA, we adopted a once a day ciclosporin microemulsion under guidance of absorption profiles (AP) in refractory NS. The blood concentrations of CSA 0, 1, 2, 3 and 4 hours after treatment (C0, C1, C2, C3 and C4), and the area under the blood-concentration-time curve of CSA from 0 to 4 hours after treatment (AUC0-4) were calculated. Based on the experience of renal transplantation, we set up the targeted optimal range of AUC0-4 was 1500 to 2000ngċhr/ml and the desired peak value (C1 or C2) as 700 to 800ng/ml. We have studied 16 patients with refractory NS and some patients with high risk for CSA therapy (range; 19-77 yo, 7 membranous nephropathy, 1 focal segmental glomerulosclerosis, 6 frequent relapsing minimal change NS and 2 mesangiocapillary prolifelative glomerulonephritis). These patients were given 2 to 3mg/kg/day of ciclosporin microemulsion once a day with low-dose prednisolone. AP was measured within a week after the initiation of single a day ciclosporin microemulsion. A fixed weight adjusted dose AUC0-4 was 2700ngċhr/ml on the average and it's range was 1187 to 5506ngċhr/ml, at the initial average dose of CSA of 2.44mg/kg/day. The average blood level of C1 and C2 were 924 and 883ng/ml, respectively. Eight of 16 patients had peak blood concentration of CSA at C1, 6 had at C2, and 2 patients had no peak level. These 2 patients seem to be a slow absorber of CSA. The average dosage of CSA declined to 1.90mg/kg/day after adjusting the dosage of CSA to achieve the target AUC0-4. The mean serum creatinine value before the CSA treatment was 0.82mg/dl (range, 0.53 to 1.32mg/dl). The serum creatinine value of the patients with higher AUC0-4 exceeding 3000 ngċhr/ml and/or higher peak value over 1500 ng/ml (C1 or C2) showed higher creatinine of 0.90mg/dl. A single daily dose CSA was well tolerated, and 11 of 16 patients were kept on complete remission and all of remaining 5 were controlled as incomplete remission. There is no refractory NS after initiation of the once a day CSA therapy guided by AP and no critical adverse events. AP guided administration of CSA is accepted as a reasonable way to achieve maximum efficacy and to minimize adverse effects in organ transplantation. Our data supported the ciclosporin microemulsion therapy guided by AP is a effective, safe and cost-saving way in the treatment of refractory NS. We assume that this method would become the standard CSA therapy in NS.

M204 THE EFFECTS OF CORTICOSTEROID THERAPY ON BONE METABOLISM IN NEPHROTIC SYNDROME

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Forty-seven nephrotic syndrome (NS) patients were involoved in the current study to evaluate the effects of high-dose corticosteroid therapy for long term on bone trunover in NS. Among them there were 28 patients treated with prednisone at the dosage of 1mg/kg/day for 6 weeks while 31 normal subjects were also studied. Serum bone gla-protein (BGP) was measured by RIA while urinary Crosslaps was measured by ELISA. The serum alkaline phosphatase (ALP), albumin (ALB), intact parathroid hormone (iPTH) and urinary protein were also measured.
The serum BGP in NS group (6.58±9.39ng/mL) was elevated in comparison with that in normal control (2.36±2.51ng/mL) but greatly declined after the treatment with high-dose prednisone (1.09±0.84ng/mL). Urinary Crosslaps excretion in NS group (1467.6±1890.7μg/mmolCr) was also elevated in comparison with normal control (356.8±240.2μg/mmolCr) but declined after treatment (495.3±687.8μg/mmolCr). The serum BGP was positively correlated with serum ALP, iPTH, urinary Crosslaps excretion while negatively correlated with serum ALB.
These findings indicate that there is a high bone turnover and negative balance between bone resorption and formation in NS due probably to the defect of the biosynthesis of 1,25(OH)2D3, proteinuria, hypoalbuminemia and elevated PTH in the serum. Bone turnover may be declined by the long-term high-dose corticosteroid treatment, leading to a better bone mineral balance.

M205 IDIOPATHIC NEPHROTIC SYNDROME IN ADULTS-PREDICTORS OF HISTOLOGY AND THERAPEUTIC RESPONSE

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Idiopathic Nephrotic Syndrome (INS) in adults is a clinical entity well known for its heterogeneity in clinical presentation, histomorphology, immunological staining pattern, therapeutic response and final outcome. Minimal Change Disease (MCD) is the commonest cause of adult INS in India and no uniform protocol is available for treatment. We conducted this study to find out the histological pattern, steroid responsiveness and prebiopsy predictors of histology in adult INS.

Study objectives were to elucidate clinicopathological spectrum and therapeutic response in adult INS and to ascertain the use of hematuria, blood pressure and serum creatinine as prebiopsy predictors. We also aim at formulating a treatment guideline for INS in adults. All patients aged 16 years or above with histology proven nephrotic syndrome were included in this prospective study conducted between January 1998 – December 2001. Immunoperoxidase staining was done on the paraffin embedded renal biopsy specimen. All patients were treated according to evidence based guidelines and followed up for atleast 2 years.

70% of all INS in adults were due to MCD and its variants. We compared the incidence of hypertension, hematuria and elevated serum creatinine at the onset in MCD and non-MCD groups. The results were analysed statistically to calculate the post test joint probability by Baye's theorem. Pre test probability of MCD is 45%. When hypertension, hematuria and elevated serum creatinine are taken in combination for predicting MCD, post test probability is increased to 85% if all are absent. The combined presence of all 3 indices reduces the probability of MCD to 0.02.

We conclude by stating that the probability of disease (MCD or Non MCD) can confidently be predicted using simple clinical and laboratory criteria. Hence an empirical steroid therapy can be safely recommended in our setting. We also propose a treatment guideline for adult INS.

M206 MYCOPHENOLATE MOFETIL IN THE TREATMENT OF CHILDREN WITH STEROID DEPENDENT NEPHROTIC SYNDROME

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Children with Steroid Dependent Nephrotic Syndrome (SDNS) are, in most istances, Cyclosporin A dependent too. We investigated the efficacy of mycofenolate mofetil (MMF) to obtain a persistent or permanent remission in children with marked SDNS. Eleven children (9 boys; mean age at the start of MMF:10.9 years) with SDNS were studied. In all patients glomerular filtration rate and arterial pressure were normal. Renal biopsy showed Minimal Change Disease (MCD) in 3 and Focal Segmental GlomeruloSclerosis (FSGS) in 8 children. MMF was started after the remission of NS was achieved with Prednisone (2mg/kg/day, maximum 60 mg/day). Patients were treated with MMF at the dose of 27.8 mg $\pm$ 4 mg/kg/day and tapering alternate day prednisone over a mean period of 9.8 months. 8 of 11 children previously received treatment with CsA (CsA dependence); in addition 5 previously received treatment also with alkylating agents or levamisole. MMF failure occurred in two patients, one with MCD and the other one with FSGS; the relapse occurred in both patients during the shift of Prednisone to alternate day regime. During the observation period the remaining 9 patients showed a sustained remission: in 4 of them Prednisone was successfully stopped, 5 are still receiving Prednisone at a mean dose of 0.3 mg/kg/day on alternate day. Two patients showed a non -nephrotic proteinuria during the 12th month of MMF treated with a transient shift of alternate maintenance dose of Prednisone to daily one. The significant reduction of mean daily steroid dose (-70% with respect to previous dose) dramatically improved the children growth velocity. No gastrointestinal or hematological side effects of MMF were seen. Our preliminary results demonstrate that children with marked SDNS due to minimal change disease and focal segmental glomerulosclerosis achieve a persistent remission during therapy with MMF, alone or in association with steroids, without significant side effects. Following therapy, a not sustained beneficial effect is reported. We have no data at the moment, but the efficacy and safety of MMF and its steroid sparing effect should be considered in preference to Cyclosporin A.

M207 EFFECTIVE AND SAFE TREATMENT OF PRIMARY NEPHROTIC SYNDROME WITH TACROLIMUS (FK 506)

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Primary glomerulopathies with protein loss are currently treated with steroids (st), cyclophosphamid (cp) or cyclosporine (cs) depending on kind of disease, patient's age and history. However, steroid refractiveness or -dependency is a common feature (10 – 30%) of albumin-loosing glomerulopathies urging the need for therapeutic alternatives. While some data are available concerning the benefits of Cs in nephrotic syndrome, there are no controlled studies on tacrolimus (Tac) in that disease. Following the comparable mechanism of Tac and it's benefits in kidney Tx, it could be hypothezid that the substance is comparable or superior to Cs in primary NS. Therefore, we conduct a clinical pilot study in steroid-refractory or -dependent primary NS to deliver primary data for a prospective, randomized trial comparing Cs and Tac in primary NS. Inclusion criteria: Men or women, age 18-70, primary nephrotic syndrome, Crea-Clerance > 25 ml/min, no response to steroid therapy or relapse after steroid taper. Treatment was conducted using 0.2mg/kg body weigth Tac twice daily targeting a serum drug through level of 5-10 ng/mL. Steroids were tapered after initial bolus of 1.5 mg/kg weight and discontinued in the longtime. ACE inhibitors and oral anticoagulants were used in all cases as appropriate.

Up to now, seven patients were enrolled exhibiting a primary protein loss of 4.0 $\pm$ 1.6 g/day. Baseline serum creatinine ranged from 45 to 250 µmol/L and did not changed significantly during therapy (week 24 137.5 $\pm$ 88.3 µmol/L). Diagnoses consisted of minimal change GN (mc, n=3), focal glomerulosclerosis (fgs, n=2), membranous nephropathy (mgn, n=1) and IgA nephropathy (IgA, n=1). 6/7 patients responded to therapy (2 completely) with an average decrease of proteinuria of 2.14 $\pm$ 0.74 g/day. Individual courses of proteinuria are given in the table. Adverse effects consisted of tremor (n=1) and fatigue (n=2). One patient discontinued the study immediately after initiation following sleeplessness and dizziness. Worsening of dyslipedemia, diabetes or other side effects were not observed.

Course of proteinuria under Tac treatment (g/day)

Patient gender/age/dignosis	baseline	week 2	week 12	week 24
m/39/mc	5.2	0.4	0.3	1.1
f/38/fgs	7.3	4.3	5	2.1
m/57/fgs	4.2	2.1	no data	no data
f/32/fgs	3.1	1.3	0.8	0.48
m/37/IgA	3.7	2.1	3.3	1.6
m/61/mgn	4.5	discontinued		
m/66/fgs	1.4	0.3	0	0.1

Thus, Tac treatment in steroid-refractory or -dependent primary nephrotic syndrome was safe and effective in this small pilot study. We are going to extend the study to 20 patients thereafter encouraging a controlled study versus Cs. Using Cs in larger studies therapy response ranged from 20 to 60%. Taking our preliminary response rate of 85% as reference we would need a population size of 49 patients in each group to prove a superior effectiveness of Tac vs Cs.

M208 REDUCED PODOCYTE EXPRESSION OF α3β1 INTEGRINS AND PODOCYTE DEPLETION IN PATIENTS WITH PRIMARY FOCAL SEGMENTAL GLOMERULOSCLEROSIS AND CHRONIC PUROMYCIN AMINONUCLEOSIDE-TREATED RATS

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Adhesion molecules, integrins, attach cells to extracellular matrix (ECM) and mediate signals from ECM to cells or from cells to ECM. They regulate cell functions, including adhesion, migration, cell cycle regulation, and differentiation. Podocyte detachment from the glomerular basement membrane (GBM) and excreted in the urine, and proteinuria are found in primary focal segmental glomerulosclerosis (FSGS), which may be associated with loss of α3β1 integrins. We have examined podocyte number in patients with primary FSGS and normal controls, and integrin α3 and β1 subunits expression of podocytes in patients with primary FSGS and chronic puromycin aminonucleoside (PAN)-treated rats by the morphometric, immunoperoxidase histochemistric and immunoelectron microscopic examination. We also measured their expression serially in rats received repeated PAN injection. The podocyte number was significantly decreased in patients with primary FSGS than controls (105±66 vs. 356±72, $p<0.05$). The immunostaining score showed that both integrin α3 and β1 subunits on podocytes in patients with primary FSGS were significantly lower than normal controls (α3 subunit: 44.8±16.4 vs. 267.1±22.2; β1 subunit: 54.5±20.1 vs. 229.1±28.3; $p<0.01$). The number of immuno-gold particles of α3 and β1 integrins at the effaced foot process area of patients with primary FSGS were also significantly decreased than normal controls (α3 subunit: 0.20±0.16 vs. 1.01±0.42; β1 subunit: 0.12±0.13 vs. 1.27±0.76; $p<0.05$). The immunostaining score of both integrin α3 and β1 subunits was negatively correlated with the degree of glomerular sclerosing score and the amount of daily protein loss, and were positively correlated with the number of podocytes. Chronic 12-week PAN-treated rats showed similar findings with decreased immunostaining expression and immuno-gold particles of α3 integrin on podocyte than normal controls (immunostaining score: 24±21.9 vs. 274.5±26.4; immuno-gold: 0.72±0.29 vs. 1.85±0.40; $p<0.05$). The chronic PAN-treated rats also showed a trend toward gradually decreased the immunostaining expression of integrin α3 subunit on podocytes during the progress from normal to FSGS state. These studies indicate that podocyte expression of integrin α3 and β1 subunits is significantly reduced in human with primary FSGS and chronic PAN-treared rats, before the morphological changes of FSGS are observed. The decreased podocyte expression of α3β1 integrins is closely related with podocyte depletion, glomerular sclerosis and daily protein loss in patients with primary FSGS.

M209 NPHS2 AND ACTN4 GENES ANALYSIS IN ADULT ONSET, NON-FAMILIAL, FOCAL SEGMENTAL GLOMERULOSCLEROSIS (FSGS)

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Mutations in *NPHS2*, encoding podocin, and in ACTN4, encoding a-actinin-4, have been identified in childhood onset familial forms of focal and segmental glomerulosclerosis (FSGS). NPHS2 may be also responsible for some sporadic cases of paediatric FSGS. The role of *NPHS2* and ACTN4 in the adult sporadic form of the disease is still unknown. We studied 33 adult subjects who had received a clinical and pathologic diagnosis of sporadic FSGS. The presence of multiplex families that presented with a clear familial inheritance for proteinuria or other congenital nephrotic syndrome was excluded. At biopsy 12 pts had Nephrotic Syndrome, 5 pts isolated proteinuria and 16 pts proteinuria and haematuria. GFR was in the normal range in 19 subjects, 14 pts had a variable degree of renal failure. The whole coding region, all intron/exon boundaries, some intron sequences (on average 50 bp on each side of the exons) of *NPHS2* and exon 8 of the *ACTN4* gene were analyzed in all patients by denaturing high performance liquid chromatography (DHPLC). DHPLC analysis was performed on an automated DHPLC instrument (Transgenomic Inc., San Jose, CA). Thirty-three DNA from FSGS patients and two normal control samples were subjected to DHPLC analysis. The eight exons of *NPHS2* gene and exon 8 of *ACTN4* with flanking intronic regions were analyzed to searching disease-causing defects.

The analysis allowed us to identify five yet described polimorphisms on the *NPHS2* gene, one undescribed intronic nucleotide change (IVS3-21C>T) and never causative mutations. In the amplicon containing exon 8 of *ACTN4* we identify the intronic polimorphis IVS7-54C>T.

NPHS2 and ACTN4 genes seem not to be involved in the pathogenesis of adult onset, non-familial FSGS. Our preliminary data do not support the need of a molecular screening of the podocin and the a-actinin-4 genes in these patients.

Free Communication June 12

M210 PERMEABILITY ACTIVITY OF FSGS SERA: INCIDENCE, PARTIAL CHARACTERIZATION AND POTENTIAL MECHANISMS

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A circulating permeability factor has been documented in sera of patients with FSGS. We have standardized a permeability assay using isolated glomeruli and a 1:50 dilution of serum or plasma. Results are expressed as P_{alb}, a dimensionless variable that ranges from 0 to 1.0. Average P_{alb} for normal sera is 0.00 ± 0.05 (mean ± SD), and for patients with ESRD due to nonglomerular diseases, 0.22 ± 0.4. We tested samples from 307 patients during clinical evaluation of renal disease between January 2000 and May 2002. The majority had biopsy-proven FSGS. Renal function varied widely and many patients were awaiting transplantation. P_{alb} values ranged from 0 to 0.99 with an average of 0.46 ± 0.28. This value is comparable to that which we have previously reported. Multiple specimens from 67 indivduals were tested prior to and after plasmapheresis or immunoadsorption, without intervening extracorporeal therapy, or before and during treatment with cyclosporine A. P_{alb} was diminished significantly in 90% of cases after extracorporeal therapy (N=16). In contrast, P_{alb} was not changed in specimens from patients who did not receive extracorporeal therapy (N=24) or in specimens obtained during or after treatment with cyclosporine A (N=27). The nature and identity of the permeability factor in FSGS are unknown. We have isolated a protein fraction that increases P_{alb} within 2 minutes and causes proteinuria after injection in rats. The apparent concentration of active protein(s) is less than 1 mg/l plasma. The protein(s) in this fraction have MW< 30 kDa, are hydrophobic, anionic and glycated. Basic amino acids constitute less than 10% of the total amino acid composition. The amino acid composition (nM/nM AA) is: Aspartic acid 0.120; Glutamic acid 0.110; Serine 0.098; Threonine 0.095; Glycine 0.094; Proline 0.088; Alanine 0.072; Leucine 0.068; Lysine 0.066; Valine 0.059; Isoleucine 0.048; Phenylalanine 0.027; Histidine 0.018; Tyrosine 0.013; Arginine 0.011; Methionine 0.005. The carbohydrate content, determined by ion exchange HPLC after acid hydrolysis, includes glucose, galactose, galactosamine and mannose. Aspartic acid, serine and threonine are likely sites of glycation. Mass spectrometry of tryptic digests reveals fragments that are shared among bands on the gel. The proteins in this fraction, as well as permeability and proteinuric activity, are absent from pooled normal plasma and from plasma obtained after therapeutic plasmapheresis. Further studies indicate that the active fraction causes glomerular release of arachidonic acid and synthesis of eicosanoids, alters the phosphorylation of cytoskeleton-associated and other proteins in glomeruli, and decreases nephrin expression. Our findings confirm the presence of a protein or family of small anionic proteins that carry permeability activity, induce proteinuria in experimental animals, and elicit responses in glomerular cells that result in impaired permeability barrier fucntion. We conclude that protein(s) in this fraction may contribute to proteinuria in patients with recurrent FSGS.

M211 PERMEABILITY ACTIVITY IS PRESENT IN SERA FROM PATIENTS WITH COLLAPSING GLOMERULOPATHY

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Collapsing glomerulopathy (CG) is a histological subtype of idiopathic focal segmental glomerulosclerosis (FSGS) that is characterized by heavy proteinuria, poor response to therapy and rapid progression to ESRD. Its incidence has increased and its etiology is unknown. Sera of patients with recurrent FSGS after transplantation impair the glomerular protein permeability barrier during *in vitro* testing. Albumin permeability (P_{alb}) of isolated glomeruli averages 0.4 after incubation with sera of unselected FSGS patients and 0.8 after incubation with sera of patients with rapid progression or recurrence in renal allograft. To test the hypothesis that CG serum possesses this activity, we measured P_{alb} after incubating isolated glomeruli with patient sera (1:50 vol/vol). By convention, we have termed $P_{alb} \geq 0.5$ as positive in testing clinical samples of individual patients. Results are shown as mean $\pm$ SD. CG was diagnosed in 14 patients from a single center using biopsies performed between June 1995 and June 2002. Sera was obtained at the latest follow-up visit. Age at diagnosis ranged from 21 to 65 years. Seven were male; 11/14 were African-American, 3/14 were Hispanic. Two patients had a family history of renal disease. Nephrotic syndrome was present at the time of biopsy in 10/14. Proteinuria at diagnosis ranged from 2.6 to 38 g/day (12.8 $\pm$ 9.3). Plasma creatinine at diagnosis ranged from 1.0 to 3.9 mg/dl (2.3 $\pm$ 0.9). Follow-up of 0.5 to 10.3 years (3.2 $\pm$ 3.7) was available for the 11 patients biopsied prior to April 2002. Plasma creatinine at follow-up in the 9 patients without ESRD ranged from 1.3 to 5.1 mg/dl (3.0 $\pm$ 1.4). Plasma creatinine had increased by $\geq$0.2 mg/dl in 8 patients, 2 of whom had progressed to ESRD. Proteinuria decreased in 11 patients after treatment with prednisone, ACE-I/ARB, CsA, cyclophosphamide or MMF. Proteinuria at follow-up ranged from 0.3 to 10 g/day (3.6 $\pm$ 3.2). Change in proteinuria in patients without ESRD ranged from +7.2 to -28 g/day (–12.6 $\pm$ 7.6). P_{alb} values ranged from 0.32 to 1.0 (0.63 $\pm$ 0.22, median 0.6); 11 were $\geq$0.5. P_{alb} of the 2 patients with ESRD were 0.72 and 0.69; one patient underwent transplantation and experienced recurrence. The distribution of P_{alb} values was significantly different from that of unselected patients with FSGS ($p < 0.01$) but was not different from that of patients with recurrent FSGS after transplantation or from a series of patients with CG we have previously described. Within the current sample of patients with CG, P_{alb} did not vary with any demographic or clinical parameter, including proteinuria or progression to ESRD. These results replicate our previous data, and confirm the generalizability of our finding that sera from nearly all CG patients increase P_{alb} and that the level of activity is higher in CG than in unselected FSGS patients. It remains to be determined if CG plasma contains an substance identical or similar to the one we have isolated from plasma of patients with recurrent FSGS.

M212 PLASMA PERMEABILITY FACTOR (S) ISOLATED FROM PATIENTS WITH FOCAL SEGMENTAL GLOMERULOSCLEROSIS REDUCE (S) NEPHRIN AND PODOCIN EXPRESSION IN HUMAN GLOMERULAR EPITHELIAL CELLS

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Plasma factor(s) (PF) displaying permeability activity in vitro and possibly determining proteinuria have been described in patients with idiopathic focal segmental glomerulosclerosis (FSGS). Indirect evidence supports the concept that PF may persist over time in patients with FSGS and induce posttransplant recurrence of the disease. We recently demonstrated that nephrin expression was reduced in patients with FSGS. In this study, we investigated the effect of PF on nephrin and podocin expression and on actin cytoskeletal organization in human cultured glomerular epithelial cells. PF were prepared from plasma eluates of patients with FSGS following a procedure based on protein A Sepharose and differential precipitation in ammonium sulphate and actually represent a partial purification product. Nephrin and podocin expression was studied by immunofluorescence microscopy and semi-quantitatively evaluated by measuring immunofluorescence intensity by digital image analysis. PF induced redistribution and loss of nephrin from the surface of glomerular epithelial cells, which was evident at 10 minutes and reversed at 24 hours. The effect of PF on nephrin expression was associated with changes in cytoskeleton distribution, including loss in stress fibers, cortical accumulation of F-actin and cell retraction and was inhibited by cytochalasin B, a compound that affects the microfilaments of the microtubular system. Moreover, it required metabolic energy, as it was prevented by sodium azide. At variance of nephrin expression, PF did not induce any change in podocin expression after 10 and 30 minutes, whereas it induced a marked reduction after 24 hours. These data indicate that nephrin and podocin expression changes induced by PF in human glomerular epithelial cells may be a potential mechanism for proteinuria in patients with FSGS.

M213 DOWN REGULATION OF PLASMA HEMOPEXIN ACTIVITY BY CORTICOSTEROIDS THROUGH MESANGIAL AND ENDOTHELIAL CELLS IN VITRO

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Previously it has been shown that stimulated endothelial or mesangial cells in vitro show enhanced ecto-apyrase (CD39) activity as compared to non-stimulated cells. These cells were able to convert non active hemopexin (Hxi) to hemopexin (Hx) with protease activity (Hxa) following incubation. [Hxi was prepared by incubation of active Hx (Hxa) with ADP (2 mM ADP/ml PBS containing 1.5 mg Hxa)]. Hxa is believed to play a role in corticosteroid responsive nephrotic syndrome (CRNS). The question emerged whether corticosteroids are able to inhibit ecto-apyrase activity of LPS stimulated endothelial or mesangial cells in vitro.

Confluent human endothelial cell cultures were incubated with or without LPS (10 ng/ml) under standard conditions. Parallel cultures were supplemented with prednisolone (pred) (2.5 mM), with or without the glucocorticoid receptor antagonist mifepristone (0.5 μM). Identical experiments were done with cultures of a human mesangial cell line. After 24 hours cytospins were prepared and stained for ecto-apyrase expression or activity by immuno- or cytochemical staining respectively. Apyrase activity of confluent cultures was assayed biochemically, using a standard assay. mRNA for CD39 of these cells was detected using RT-PCR. Finally the activity of soluble apyrase (0.16 U/ml) was tested biochemically with or without supplementation of pred (2.5 mM) for its phosphatase activity.

LPS-stimulated endothelial cells showed significantly decreased ecto-apyrase expression and activity after treatment with pred as compared to non pred-treated cells. Simultaneous incubation of mifepristone did not inhibit the effect of pred. Identical results were found in experiments with mesangial cells in vitro. Mean values of pred-treated versus non pred-treated cells from the biochemical assay: endothelial: 0.14$\pm$0.01 mM PO_4 vs 0.20$\pm$0.005 mM PO_4;$P \leq 0.05$ and mesangial: 0.04$\pm$0.002 mM PO_4 vs 0.08$\pm$0.004 mM PO_4; $P \leq 0.05$]. RT-PCR results showed identical mRNA signals for apyrase in pred non pred-treated cells. Interestingly soluble apyrase showed a significant decrease of activity following preincubation with pred, [50% vs non-treated apyrase; $P < 0.01$].

The significant down regulation of cellular ecto-apyrase activity in LPS-stimulated cells in vitro due to pred may potentially inhibit the conversion of Hxi to Hxa by activated endothelial or mesangial cells. Since Hxa is a potent pro-inflammatory mediator, the present activity of pred may reflect a novel anti-inflammatory principle. This particular pred effect (which occurs also when dexamethasone is used instead of pred) is probably directed towards ecto-apyrase in a non genomic manner, as both gene transcription for CD39 seems not altered, whereas also soluble apyrase activity can be down regulated by pred in vitro. Also the non involvement of the cytosolic glucocorticoid receptor, which is thought to operate mainly via genomic pathways, supports this hypothesis. The intriguing possibility that this form of corticosteroid action may also play a role in Hx activation of CRNS in vivo is currently under investigation.

M214 HUMAN MESANGIAL CELLS EXPRESS HEMOPEXIN ON THEIR CELL MEMBRANES FOLLOWING ACTIVATION WITH TNFα IN VITRO

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It has been shown recently that primary human mesangial cell cultures were able to release hemopexin (Hx) after stimulation with cytokines in vitro. The active isoform of this molecule (Hxa), showing protease activity, is thought to play a role in the pathogenesis of proteinuria in minimal change disease (MCD). Although western blots of concentrated mesangial cell culture supernatants as well as immunostaining of cytospins of these cytokine stimulated cells clearly showed the presence of this molecule, we were not able to detect whether mesangial Hx exerts functionally identical protease activity as compared with plasma Hxa.

Therefore we now incubated cells from a human mesangial cell line ($2x10^6$cells/ml PBS, pH 7.2) with (n=4), or without (n=3) TNFα (10ng/ml) with unfixed (rat) kidney tissue at 37°C. Parallel cultures (n=4) were set up in which the culture medium was supplemented with either monoclonal anti Hx IgG (0.15mg/ml) or monoclonal anti albumin IgG (0.15mg/ml) or ATP (2mM). After 2 hours the incubation was discontinued, sections were washed with culture medium (RPMI 1640) and stained for glomerular ecto apyrase by immunostaining according to standard methods. The decrease of glomerular reaction product was considered as a standard for protease activity of Hx. The reaction product in glomeruli was quantified by computerized image analysis.

The results show significant loss of glomerular ecto apyrase exclusively in kidney sections after contact with TNFα stimulated mesangial cells. Kidney tissue following incubation with non stimulated cells or TNFα stimulated cells in the presence of anti Hx IgG or ATP did not show affection of glomerular ecto apyrase. In contrast to anti Hx IgG, anti albumin antibody did not inhibit the effect of TNFα stimulated cells upon glomerular ecto apyrase.

Since it has been shown that both ATP as well as anti Hx IgG specifically inhibit Hxa, it is likely that the apyrase affecting activity of stimulated mesangial cells is mediated through a membrane associated Hxa molecule.

It is concluded that human TNFα stimulated mesangial cells are potentially able to produce an active isoform of Hx. The possible in vivo relevance of local intra glomerular Hxa production by stimulated mesangial cells in relation to T cell cytokines and proteinuria in MCD, remains to be established.

M215 GENETIC MARKERS FOR PROGRESSION OF FOCAL-SEGMENTAL GLOMERULOSCLEROSIS TO UREMIA

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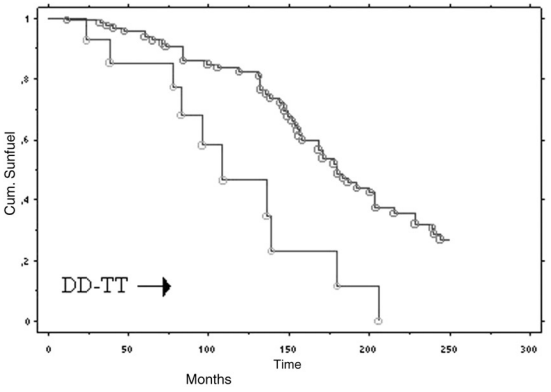
FSGS is becoming a primary cause of uremia. Mutations of podocyte genes involved in familial FSGS such as NPHS2 have emerged as primary causes of sporadic FSGS with rapid evolution to end stage renal failure (ESRF). Moreover, genetic variability linked to the renin-angiotensin system (ACE, Agt, AT1) and related molecules involved in recruitment of inflammatory cells (osteopontin, OPN), removal of matrix (PAI-1), or with dilating effects (bradykinin 2 receptor, B2BKR) have been proposed as secondary markers for evolution. To define these putative associations, 273 patients with primary nephrotic syndrome were first screened for mutations of the relevant podocyte genes NPHS1, NPHS2 and a.actinin 4; 24 presented homozygous/composite heterozygous/single mutation of podocin and had progression to uremia. The remaining 249 were genotyped for the following polymorphisms and SNPs: ACE D/I, Agt M235T, AT1 A1166C, PAI-1 4G/5G, OPN TG/TGTGintr1 and B2BKR –9/+9. Of this cohort 154 pts had steroid resistance and a histology compatible with FSGS; 62 had progression to ESRF.



The ACE DD genotype was more frequent in progressors (55%) than in non progressors (37%) ($p<0.05$), while genotypes of other molecules were comparable. The cumulative survival, analysed by Kaplan Meier model, was worse for ACE-DD who carried the Agt-TT genotype, that is associated with high levels of angiotensinogen (Logrank p =0.0007). A comparably worse progression to ESRF was found in pts with the B2BKR +9/+9 genotype (that is associated with low bradykinin receptor expression and high vasoconstriction) stratified for the Agt-TTgenotype (p=0.02).

These data demonstrate that evolution of FSGS to ESRF is strongly influenced by genetic conditions associated with high expression of molecules of the RAS system with an addictive effect of ACE-DD and Agt-TT polymorphisms. At the same time, evolution is influenced by high renal vaso-costriction, that is genetically determined by the B2BKR genotype. However, progression is mainly influenced by primary genetic conditions related to mutations of podocin that must be excluded before looking at other markers.

M216 COAGULATION FACTOR GENES POLYMORPHISMS IN PRIMARY SPORADIC FOCAL SEGMENTAL GLOMERULOSCLEROSIS

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Focal segmental glomerulosclerosis (FSGS), a common cause of nephrotic syndrome (NS), is responsible of about 10% of the end-stage renal disease (ESRD) in adult population. Nephrotic syndrome is frequently accompanied by hypercoagulability, mainly due to urinary loss of antithrombin III. Thrombophilia in NS is a mortality and morbidity factor and is related to the progression of renal disease.

In order to assess the role of coagulation factors in FSGS and steroid response, we evaluated the Factor II, Factor V Leiden and methylenetetrahydrofolate reductase (MTHFR) Single Nucleotide Polymorphisms (SNPs) in patients with primary FSGS. We selected a cohort of 40 adult patients with FSGS biopsy proven and a sample of 100 healthy control people, both of Italian ancestry. In these populations we tested, using SNaPshot ddNTPs Primer Extension kit on a ABI Prism 310 Genetic Analyzer automated sequencer (Applied Biosystems, Foster City, CA), the following SNPs: a common mutation within the factor V (G1691A), a common mutation in prothrombin (FII) gene (G20210A), and a common polymorphism in MTHFR gene (C677T). Frequency differences were evaluated by the chi-squared and Fisher exact test.

G1691A and G20210A mutations were present in heterozygote status in 1 patient for each SNP (frequency 2.5%), and in 5 (5%) and 3 (3%) controls respectively, apparently concordant with the prevalence of general population.

The MTHFR SNP C677T was present in 42 out of 80 chromosomes (52.5%) in FSGS patients and in 82 out of 200 control chromosomes (41%). No differences were seen in genotype frequencies between the two groups. 26 FSGS patients underwent steroid therapy: 13 resulted resistant and 13 responsive.
The proportion of steroid resistant patients was higher among those carrying TT genotype than those carrying CT and CC genotype (Fisher exact test, p=0.007).
Conclusions: 1) 677T allele of the MTHFR gene does not seem to be associated to idiopathic FSGS; 2) TT genotype resulted strongly associated with steroid resistance in this group of patients suggesting a predictive value of the response to corticosteroid therapy.

M217 IDIOPATHIC FOCAL SEGMENTAL GLOMERULOSCLEROSIS: AN IMPORTANT CAUSE OF END-STAGE RENAL FAILURE IN THAILAND

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Focal segmental glomerulosclerosis (FSGS) is one of major causes of idiopathic nephrotic syndrome in Thailand. Thirty to forty percents of those patients develop ESRD after 5–15 years. The most important prognostic factor is the response to steroid treatment. In this study, we retrospectively analyzed 70 adult patients who had biopsy-proven idiopathic FSGS. The prevalence of FSGS among primary glomerular diseases was 14.1%. The indications for biopsy in most patients were steroid-resistant or relapsed nephrotic syndrome. The ratio of male and female patients was 39:31, and the average age was 33.3 (range 15–67) years old. Mean blood pressure was 141.1/90.7 mmHg (71% of the patients had hypertension), BUN 31.6 mg/dl, serum creatinine 2.4 mg/dl, serum albumin 2.4 g/dl, serum cholesterol 371.2 mg/dl, and urine protein excretion 5.0 g/24 hours. Most patients were treated with oral prednisolone with/without cyclophosphamide. Hypertension was mostly treated with ACE inhibitors. Nineteen patients (27%) had complete remission (CR), ten (14%) with partial remission (PR), eleven (16%) had PR with steroid-dependent, and twenty-three (33%) did not respond to treatment. Seven patients (10%) had progressive renal insufficiency and developed ESRD within 6 months, one of whom had renal transplantation. The patients with complete or partial remission had stable renal functions during follow-up period. One patient developed slowly progressive renal failure after 7 years of follow-up. This patient had normal initial renal function and severe hypertension, but did not respond to treatment. In conclusion, FSGS is a common primary glomerular disease in Thailand. Most of the patients had hypertension. Treatment with prednisolone with/without cytotoxic drugs induced remission in 57%. Ten percents of the patients with initial renal dysfunction had progressed to ESRD within 6 months. Patient who had severe hypertension and did not respond to treatment may develop progressive renal failure several years after the onset of the disease.

M218 CLINICAL AND PATHOLOGICAL FEATURES OF OBESITY-ASSOCIATED FOCAL SEGMENTAL GLOMERULOSCLEROSIS

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Obesity is defined as a body mass index (BMI) greater than 28kg/m^2 according to Chinese diagnostic criteria. Fifteen obesity-associated focal segmental glomerulosclerosis cases (OB-FSGS) and fifteen non-obese (BMI<24kg/m^2) idiopathic FSGS cases in our division during the past 4 years were reviewed, and their clinical, laboratory and pathological features were analyzed.]
The clinical and laboratory data of OB-FSGS and I-FSGS are listed in Table. Pathlogically, glomerular diameter in OB-FSGS (232.43±24.77μm) was significantly larger than that in I-FSGS (176.49±28.65μm, P<0.01).
In conclusion, patients with OB-FSGS usually have metabolic disorders, a lower incidence of nephrotic syndrome despite nephrotic-range proteinuria, more slowly progressive course, and glomerulomegaly besides FSGS in pathological examination. So, OB-FSGS is distinct disease entity from I-FSGS.

The clinical and laboratory data of OB-FSGS and I-FSGS

	OB-FSGS	I-FSGS
Clinical manifestations:		
Couser (months)	26.68±46.72	10.13±14.24
Nephrotic syndrome	0/15 **	9/15
Hypertesion	11/15	9/15
Complications:		
Glucose intolerance	5/15 *	0/15
Hyperuricemia	2/15	0/15
Hypertriglyceridemia	14/15 **	3/15
Laboratory data:		
Urine protein (g/d)	3.57±2.79	5.21±2.97
Serum albumin (g/L)	39.15±2.61 **	23.60±0.26
Creatinine clearance (ml/min)	81.19±27.05 **	46.22±39.17

* p<0.05,** p<0.01

M219 PREDICTING RENAL SURVIVAL IN IRANIAN CHILDREN WITH FOCAL SEGMENTAL GLOMERULOSCLEROSIS

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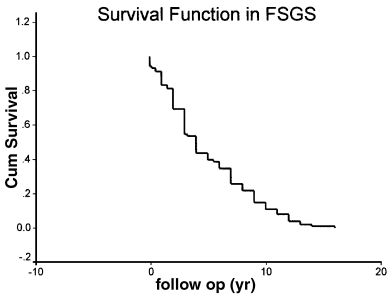
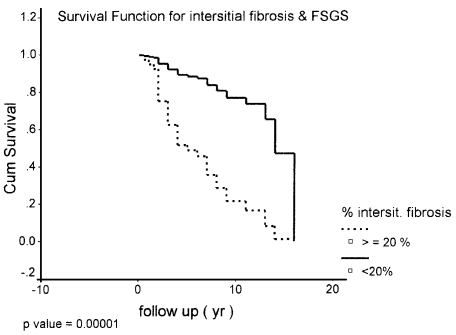
103 children between ages 6 months to 14 years under diagnosis of FSGS, that refered to Ali asghar Hospital and Dr Ahari Childrens Hospital from 1984 until 2000, were reviewed retrospectively. Demographic data,clinical signs and symptoms at the time of diagnosis, lab data,histopathologic findings, immunofluoroscent findings and response to prednisolon were evaluated. Also renal survival and its relation to the mentioned factors determined.
Results: The actual renal survival at 1-5-10 and 15 years were 97.9%-72%-47% and 17% respectively and reached to 12% at the end of this study (16 years). The median renal survival was 9.04 ±1.8 years. The hazard rate increased after 5 years of disease. In univariate analysis by the Kaplan-Meier method the variables that significantly associated with poor outcome were: initial low GFR, hypertention, anemia, renal injury score more than 30%, interestitial cellular infiltration, cortical intrestitial fibrosis more than 20%, peritubular and peri glomerular fibrosis, tubular atrophy and interestitial edema. But using back ward multivariate cox model, the only variables that remained independently with adverse outcome were interestitial fibrosis equal or more than 20% (p value=0.00001) and hypertention (p=0.03).





Conclusion: Using more agressive treatment for patients with interestitial fibrosis more than 20% and better controle of hypertention may improve the outcome of Iranian children with Primary FSGS.

M220 EVALUATION OF STEROID RESPONSE IN PRIMARY FOCAL SEGMENTAL GLOMERULOSCLEROSIS (A SINGLE CENTER STUDY IN IRAN)

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Primary focal segmental glomerulosclerosis (FSGS) is one of the leading causes of renal failure and end-stage renal disease in adult nephrotic patients. Immunosuppressive therapy may change its course. Serum creatinine level and degree of proteinuria are significant prognostic factors. We conducted a cross sectional study to evaluate steroid response in biopsy proven FSGS patients. Sixty patients were included in the study. Thirty –seven were male and 23 female. Mean age of patients was 34.5 ± 10.8 years. Mean duration of follow up was 2.9 ± 2.1 years. Presenting features were as follows; proteinuria 100%, hypertension 48.3%, hematuria 16.7%, elevated serum creatinine, higher than 1.3mg/dl, 46.7%, hypercholestrolemia 45% and hypertriglyceridemia 43.3%. Proteinuria was subnephrotic in 28.3% of patients. Patients were treated by prednisolone with or without ACE inhibitor. The duration of steroid therapy was about 6 months. Response to treatment was defined as stable level of serum creatinine and complete remission (proteinuria < 250mg/d) or partial remission (proteinuria 0.26-2.5g/d). Complete remission of proteinuria occurred in 32.7%, partial remission in 30.9% and no response in 36.4% of patients. Response to treatment was observed in 58.8% of patients with normal serum creatinine and in 8.3% of patients with serum creatinine over than 1.3 mg/dl. At the end of study, CRF and ESRD developed in 34.5% and 16.4% of patients,respectively. In this study there was significant correlation between serum creatinine level at the time of diagnosis and the response to treatment ($p<0.05$). There was no significant correlation between degree of proteinuria and renal outcome. Many uncontrolled studies have found that nephrotic adults with FSGS have a remission rate between 35-60% when treated by prednisolone for 4 months or more.
The results of this study also confirmed that steroid therapy for about 6 months has a good response in adult FSGS patients.

M221 STEROID RESPONSE IN PRIMARY FSGS: DOES BASELINE FEATURES MATTER

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Nephrotic Syndrome (NS) secondary to Primary Focal Segmental Glomerulo Sclerosis (FSGS) is steroid responsive in a proportion of patients. We have retrospectively analysed response to steroid and outcome in patients with primary FSGS presenting with NS at our center.
Between Jan. 1994- - Dec.2001, out of 730 patients presenting with NS 122 (16.7%) had biopsy proven FSGS. Eighty-three had complete data and follow-up, 51 male and 32 female, with a mean age of 25.21± 9.707 (range 14-60 years). Eleven received steroid in uncertain dose and duration outside and were not re-treated. Seventy-two patients received oral steroid at starting dose of 1 mg/kg/day tapering to 0.75 mg/kg/day at 6 weeks or earlier in case of response. This dose was given for subsequent 6 weeks and then tapered.
Thirty-five (48.6%) patients responded to steroid therapy and became proteinuria free after a median interval of 4.8 weeks (1-11weeks). Within a minimum follow-up of 12 months seven had relapsed. Duration of nephrotic oedema and baseline proteinuria values were similar in steroid responsive and non-responsive group, 15.94 vs 15.37 months and 7.9 vs 5.8 grams/day respectively. Response to steroid was not blunted by elevated serum creatinine as proportion of patients with serum creatinine > 1.5 mg/dl was 31.4% among steroid responsive group vs 10.8% in non-responsive group. Hypertension was the only factor negatively correlating with steroid response. 40% vs 70%hypertensives in responsive and non responsive group respectively.After a mean follow-up of 28.11± 21.19 months seven patients developed chronic renal failure all were either non-responsive or belonged to the group of non- treated patients.
In conclusion baseline features have little influence on steroid response in FSGS. Steroid treatment should therefore be employed in all FSGS patients as remission from proteinuria and preservation of renal function is achieved in nearly 50% of cases.

M222 ONE- YEAR SURVIVAL AND RENAL FUNCTION OUTCOME IN PATIENTS TREATED BY PLASMAPHERESIS FOR GOODPASTURE SYNDROME: A 73 CASE REPORT STUDY

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This retrospective multicenter study (over the last 20 years) describes patients with Goodpasture syndrome treated by plasmapheresis included in the Registery of the Société Française d'Hémaphérèse, and their one-year outcome. Seventy three patients (51 men, mean age 36.9 ± 19.6 years) were included in this study. At presentation, 76% had pulmonary haemorrhage and median serum creatinine level was 630 µmol/l. Anti-glomerular basement membrane (GBM) antibodies were found in the serum of 59 out of 67 patients, i.e. 88%. Renal biopsy was performed in 69 patients (94%) and disclosed crescentic diffuse glomerulonephritis, and linear glomerular IgG deposits in 67 patients (97%). There was a positive correlation between: i) the initial serum creatinine and the number of crescent (p=0,001) and ii) the young age and the occurrence of lung haemorrhage (p=0.002). All patients were treated by plasmapheresis (median: 12 sessions) and intravenous and/or oral corticosteroids. In addition, 83% of the patients received either by intravenous or oral cyclophosphamide (respectively, 53% and 30%). 66% of the patients needed extra-renal epuration during the first three months. One-year patient survival was 90.3% (Kaplan-Meyer). Age greater than 60 years old was significantly associated with an increased mortality. At one year, 6 patients (8%) were lost of follow-up, 15 patients (20%) had favorable renal evolution (median creatinine level at 100 µmol/L) (group I) and 52 patients (71%) had detrimental outcome (7 deaths, 42 chronic dialysis and 3 renal transplantation) (group II). There was a negative correlation between both serum creatinine level (p=0.001) or the number of extra-capillary crescents (p=0.001) and renal function outcome. At baseline, serum creatinine level and the number of extra-capillary crescents were significantly lower in group I as compared to group II (respectively, 320 vs 830 µmol/l, $p<0.001$ and 28 vs 97%, $p<0.001$). In multivariate analysis the only significant factors that influence long-term renal outcome were serum creatinine level ($p = 0.001$) and the number of crescent at the initial biopsy ($p = 0.001$).
In conclusion, in Goodpasture syndrome, agressive immunosuppressive treatment including plasmapheresis decreases patients' mortality but renal function outcome remains poor. *Free Communication June 9*

M223 MACROPHAGE INFILTRATION IN CHRONIC GLOMERULONEPHRITIS: THE ROLE OF PROTEINURIA, MCP-1/CCL2 AND CCR2

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We hypothesised that tubulo-interstitial (TI) macrophage (MØ) infiltration in human glomerulonephritis (GN) plays a central role in directing injury at this site. Further, that MØ recruitment was directed by proteinuria induced tubular monocyte chemoattractant protein (MCP-1) secretion thus explaining the deleterious effect of proteinuria on renal function. We therefore investigated the role of MCP-1/CCL2, its receptor CCR2, and proteinuria in the recruitment of MØ to TI sites in patients with chronic GN.
Prospectively collected renal biopsy tissue and early morning urine samples from 59 patients with GN (calculated creatinine clearance (CrCl) median

83.5 ml/min (range10-134), urinary albumin/creatinine ratio (ACR) median 197.3 (0.5–1669) were studied. TI macrophages, stained immunohistochemically (IHC) with monoclonal anti-CD68 antibody, were quantified (mean number in 10 randomly selected high power fields (HPF)). Dual staining IHC with monoclonal antibody to CCR2 was used to analyse MØ expression of CCR2. Urinary MCP-1 was quantified by ELISA. Cultured human proximal tubular epithelial cells (PTECS), when confluent in T25 flasks, were incubated for 24hrs with human serum albumin (HSA)(0.1, 1 and 10mg/ml) and supernatant MCP-1 measured by ELISA.
MØ infiltrates at TI sites in GN (mean 49.4/HPF (SE 3.03)) was often localised to tubular epithelial cells. In patients with CrCl>70mls/min (n=38) MØ numbers increased with proteinuria (mean 32/HPF, ACR<150; 41/HPF, ACR150-400; 53/HPF, ACR > 400; ANOVA, p=0.01). Urinary MCP-1 levels correlated with the degree of proteinuria (R=0.54,p<0.01). Infiltrating MØ expressed CCR2 and numbers correlated with urinary MCP-1 levels (R=0.54, p<0.01)and inversely with creatinine clearance (R=-0.59, p<0.01). In vitro, HSA stimulated dose-dependent, PTEC MCP-1 production with a maximum of 3537 pg/mg cell protein.
These results show that proteinuria stimulates tubular cell production of MCP-1. The correlation between urinary MCP-1 and urinary protein on the one hand and TI macrophage infiltration on the other indicate a role for proteinuria in MCP-1/CCR2 dependent TI macrophage recruitment in human chronic GN.

M224 EXPRESSION OF THE CHEMOKINE RECEPTOR CXCR3 IN HUMAN GLOMERULONEPHRITIS

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Chemokines play a pivotal role in the recruitment of specific inflammatory cells into the kidney. The chemokine receptor CXCR3 expressed on activated lymphocytes has a key role in guiding T cells to sites of injury. In addition, data have been provided for an potential expression of CXCR3 by human mesangial cells.
To determine the distribution of CXCR3 expressing cells in human glomerulonephrits we performed immunohistochemistry using a monoclonal antibody (1C6, BD Pharmingen) on formalin-fixed, paraffin-embedded tissue. A total of 48 renal biopsies from patients with IgA nephropathy (n=26), lupus nephritis (n=12), membranoproliferative glomerulonephritis (n=7) and focal necrotizing glomerulonephritis (n=3) were evaluated. Consecutive sections were stained for CD3 positive T cells, and CCR5 positive cells. Furthermore, CXCR3 expression was studied in vitro on cultured human mesangial cells.
In human glomerulonephritis a significant part of the interstitial infiltrating cells was CXCR3 positive, whereas the number of CXCR3 positive cells in glomerular tufts was low. The distribution of CXCR3 positive cells was consistent with CD3 positive T cells. A morphological and numerical correlation between CD3, CXCR3 and CCR5 indicated a high percentage of double positive T cells in the interstitial infiltrates. The number of CXCR3 positive cells rose significantly with the percentage of interstital area involved, the percentage of globally sclerosed glomeruli, and was higher in patients with extracapillary proliferation.
Consistent with the immunohistochemistry data both PCR analysis and RNase protection assays revealed that human mesangial cells did not express CXCR3-specific mRNA even after stimulation with various proinflammatory cytokines. Under the same conditions no CXCR3 protein was detectable by flow cytometry on mesangial cells using the monoclonal antibody 1C6.
In conclusion, in human glomerulonephritis the majority of CXCR3 positive cells were infiltrating, interstitial lymphocytes, many of which were CXCR3 and CCR5 positive. CXCR3 and CCR5 positive T cells might play an important role during tubulointersitial injury and progression of glomerular diseases.

M225 THE EXPRESSION OF PLATELET-DERIVED GROWTH FACTOR RECEPTOR ALPHA (PDGF-Rα) AND ITS LIGAND PDGF-AA IN HUMAN GLOMERULONEPHRITIS (GN)

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Many research data suggest a key role for PDGF-Rα and its ligand, PDGF-AA in atherosclerosis and hypertension. On the other hand, in mice with the experimental GN, deficiency in PDGF-Rα in mesangial cells leads to an abnormal restoration of the glomerular structure. Thus, the actual relevance of PDGF-AA/PDGF-Rα signalling to the development of glomerular pathology is not clear. We looked for the expression of PDGF-Rα and PDGF-AA/AB in renal biopsies from 40 patients with different forms of GN by means of the RT-PCR and immunohistochemistry (IHM). Antibodies to the extracellular and intracellular tails of PDGF-Rα were used. Normal appearing renal tissue surrounding the removed renal tumours served as controls (N=7). The mRNA expression of the above genes was related to that of the GAPDH used as a housekeeping gene.
At the mRNA level, the expression of PDGF-Rα did not differ between the normal and diseased renal tissue. When compared to the normal kidneys, higher levels of mRNA for PDGF A-chain were observed in GN (1.22±0.21 v. 0.83±0.19), particularly in cases with non-proliferative forms of the disease (1.54±0.47). By IHM, an abundant expression of PDGF-Rα could be demonstrated on glomerular endothelial cells in the normal kidney. In contrast, the expression of PDGF-AA/AB protein was relatively low in the normal glomeruli, but also confined to the endothelial cells. In GN, a moderate increase in the expression of PDGF-AA/AB was found in non-proliferative and minor proliferative lesions. Marked to fulminant mesangial cell proliferation was associated with a dramatic loss of staining for PDGF-Rα (particularly for the extracellular part of the protein) and a sustained, although very variable, expression of PDGF-AA/AB.
Our results seem to confirm the experimental data indicating that deficiency in PDGF-Rα may be involved in the development and/or progression of human GN.

IMPAIRED ACTIN FILAMENT REMODELLING PREDISPOSING TO GLOMERULAR DAMAGE IN GLOMERULONEPHRITIS

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Chronic glomerulonephritis (CGMN) is a common cause of end-stage renal failure worldwide. Neutrophils are an important component of the inflammatory infiltrate into the mesangium and adhere via integrin, CD18/CD11b. CD18/CD11b association with the actin cytoskeleton is important for regulation of adhesion. The aim of this study was to investigate the hypothesis that actin polymerisation in relation to neutrophil CD18/CD11b expression is abnormal in CGMN patients.
Neutrophils in whole blood from normal controls (NC) and CGMN patients (Wegners granulomatosis (WGN), Membranoproliferative glomerulonephritis (MPGMN), Measngioproliferative glomerulonephritis (MSPGMN) and Systemic lupus erythematosis (SLE) were activated with phorbol ester, stained for filamentous actin with phalloidin FITC and CD11b and analysed by flow cytometry. The role of calpain and protein tyrosine phosphatases were investigated using the inhibitors E64d and phenylarsine oxide (PAO) respectively.
Following initial expression the percentage of cells losing surface CD18/CD11b was lower in CGMN patients than NC (26.1% vs 42.7%, p<0.0001) and even lower in CGMN patients with progressive disease (prteinuria; >1g/24hrs, hypertension and renal impairment) (22.4%, p<0.0001). Only neutrophils with increased actin polymerisation lost surface CD18/CD11b.
E64d reduced the proportion of cells losing surface CD18/CD11b in NC (18.5% vs 42.7%, p<0.0001) but had no significant effect in neutrophil from CGMN patients. PAO reduced the proportion of cells losing surface CD11b in all groups (NC, 36.7% vs 42.7%, p<0.05: CGMN 20.7% vs 28.2%, p<0.01)
In CGMN, impaired neutrophil actin polymerisation leads to persistent ex-

 # Author declined to present

pression of CD18/CD11b, which contributes to the pathogenesis of renal disease by increasing neutrophil endothelial adherence and mesangial influx. Calpain and protein tyrosine de-phosphorylation play a role in actin polymerisation and CD18/CD11b down-regulation. Calpain may be ineffective in CGMN patients.

M227 INTERACTION OF ANGIOTENSIN CONVERTING ENZYME (ACE) INSERTION/DELETION (I/D) POLYMORPHISM AND ANTIPROTEINURIC EFFECTS OF LOSARTAN IN PATIENTS WITH NONDIABETIC CHRONIC RENAL DISEASE

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The ACE I/D polymorphism has been suggested to play an important role in the individual response to ACE inhibition, even though the data conflict. In chronic proteinuric nephropathies, patients homozygous for the D allele have been reported to exhibit reduced antiproteinuric response to ACE inhibition. We performed this study to ascertain whether angiotensin II receptor antagonist (ARA) might overcome the interaction between ACE gene polymorphism and ACE inhibition, and induce different antiproteinuric effects among genotype groups in nondiabetic proteinuric glomerulonephritis (GN) patients. Ninty-nine nondiabetic chronic GN patients with hypertension and urine protein excretion greater than 500 mg/day were enrolled. ACE genotype distributions [II;ID;DD: 36 (36.4%); 52 (52.4%); 11 (11.2%), respectively] were similar to background Korean population. After screening assessment, current antihypertensive medications, other than alpha- or beta-blockers and/or diuretics, were discontinued during the 4 weeks of washout period and the patients received losartan 50 mg daily followed by 100 mg in two treatment periods each lasting 12 weeks. Additional antihypertensive agents other than ACE inhibitors, ARA, and calcium channel blockers were prescribed to achieve goal of 125/75 mmHg during active treatment period. At baseline and in the end of the each treatment periods, 24-hour urinary protein excretion, and creatinine clearance (CCr) values were determined. At baseline, urine protein excretion, geometric mean (range), and CCr (mean±SD) values were similar among the genotypes [II; ID; DD: 1,820 (805 to 6,684); 1,995 (719 to 9,382); 1,585 (617 to 6,266) mg/day, 63.1±25.0; 66.0±25.1; 67.4±28.6 ml/min/1.73m^2, respectively]. Both systolic and diastolic blood pressure at baseline were also not different (II; ID; DD: 136/87; 134/87; 135/89 mmHg, respectively). Both doses of losartan significantly decreased proteinuria ($p<0.05$ vs. baseline) and losartan 100 mg was more effective than 50 mg in reducing proteinuria, 51.8% (95% CI; 45.9 to 57.7) versus 40.0% (33.4 to 46.6), respectively ($p<0.01$). Among the 3 genotypes, there were no differences in the degree of ARA-induced proteinuria reduction and mean arterial BP reduction at the end of the study [II; ID; DD: 50.8% (39.7 to 61.8); 53% (44.1 to 61.1); 49.9% (34.9 to 64.8), and 13.1% (10.0 to 16.2); 10.5% (9.0 to 13.8); 14.8% (8.7 to 16.8), respectively, p=NS]. Serum creatinine and other biochemical parameters did not change significantly during the study. Dietary protein intake estimated by nPNA also remained stable (1.2 ±0.2 g/kg/day).

These results suggest that losartan, an ARA, might overcome the interaction between ACE I/D genotype and ACE inhibition, and induce similar antiproteinuric and BP lowering effects in proteinuric nondiabetic GN patients regardless of ACE I/D genotype.

M228 MAST CELLS ARE IMPORTANT CELLULAR COMPONENTS IN CHRONIC NEPHROPATY

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The progression of renal diseases is characterized by glomerulosclerosis and tubulointerstitial fibrosis. In this context, inflammatory of cells capable of producing inflammatory mediators and components of extracelular matrix are of relevance in the development of fibrosis. Recently, mast cells have been implicated in chronic inflammatory process and in the development of interstitial fibrosis. It has been demonstrated that mast cell tryptase induces proliferation and production of extracelular matrix components in fibroblasts, and that mast cell chymase has chemotactic effects in inflammatory cells. Moreover, chymase can alternatively convert angiotensin I to angiotensin II, which is important in the fibrogenic process. The aim of the present study was to evaluate the presence of mast cells in biopsies obtained from different nephropathies. Patients were classified into five groups, according to clinical and histological criteria: focal segmental glomerulosclerosis (FSGS), membranoproliferative glomerulonephritis type I (GNMP I), IgA nephropathy (IgA-N), lupus nephritis and malignant hypertension. Additional normal renal tissue obtained from kidney donors was used as control (Normal; n=12). Additionally, biopsies from cases of FSGS obtained 12 months after treatment with cyclosporin and ACE inhibitor (CsA + ACEi) were analyzed. Immunohistochemical techniques using paraffin sections were employed to identify mast cells tryptase and chymase expression.

Results (mean±SEM; * $p<0.05$ tryptase vs normal kidney)

Mast Cell	Normal kidney	FSGS	GNMP I	IgA-N	Lupus nephritis	Malignant hypertension
Tryptase (cells/mm^2)	1.8±1.3	8.5±6.2*	10.7±10.0*	17.9±14.9*	11.5±12.3*	14.4±7.1*
Chymase (cells/mm^2)	0.6±0.4	5.2±3.4	4.1±4.0	1.6±2.0	2.9±3.9	0.6±0.5

Only few tryptase and chymase positive cells were observed in normal kidney. In contrast, a significant number of mast cell tryptase positive cells was observed in all nephropathies compared to normal kidney. Mast cell chymase expression was significantly higher only in GNMP I. Mast cells were detected particularly in areas of interstitial fibrosis. In some cases, mast cells were detected within the glomeruli and tubules.

In FSGS, treatment with CsA+ACEi induced an improvement of clinical and laboratorial parameters and a decrease in the number of positive tryptase mast cells (from 8,5±6,2 to 5,0 ±5,5cells/mm^2) and chymase mast cell (from 5,2±3,4 to 2,0 ±2,7cells/mm^2)

The increased number of mast cells tryptase in chronic nephropathies suggest that these cells may be involved in the development of chronic inflammatory process.

M229 THE SIGNIFICANCE OF ENDOGENOUS OUABAIN VARIANCE IN GLOMERULAR DISEASES OR WHEN RENAL FUNCTION DECREASE

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Background and Objective: Endogenous ouabain (EO), a kind of new found Na^+-K^+-ATPase inhibitor in body of human and mammals, have the functions of adjusting the H_2O-Na metabolism and the contraction of the heart and blood vessels. This objective of investigation was the concentration of EO checked to seek the variance law of hypertension and glomerular diseases, then investigated the interface about blood pressure, renal function.

Method: The density of EO were assayed in 16 patients of hypertension (stage III, no renal function decrease), 46 patients of glomerular diseases and 20 patients as controllers. Then the EO concentration was checked used ELISA coating buffer antigen and competition for serum samples.

Result: The concentration of EO in the control group are 0.87±0.35 ng/ml. The patients suffering hypertension is higher level of EO more than the control group. The renal function normal, azotemia and uremia groups, whose EO are difference and all of 3 groups were higher. It exists no difference between the EO concentration of the acute and chronic renal failure. The normal group was subdivided into blood β_2-MG normal or increased groups, urineβ_2-MG normal or increased groups which their density are higher more than control group but themselves between normal and increase group no difference. The result indicates that EO increases as soon as the glomerular pathologic changes occur while the β_2-MG, the most sensitive index up to now, hasn't changed. The EO of primary or secondary renal diseases is no significant difference that indicates the etiology does not affect the EO level. The same result happened in different renal diseases with different pathological changes.

Conclusion: 1. The EO level of the glomerulonephritis is significant higher more than the control group. It's level has a direct relationship with the blood pressure and a reverse relationship with the renal function, whereas has no relationship with the pathological type (beside sclerosing glomerulonephritis). 2. The EO level does nothing with the hematuria, edema and mass proteinuria. About organic lesion of glomerulonephritis, serum EO is sensitive more than blood and urine β_2-MG.

M230 THE ROLE OF MATRIX METALLOPROTEINASE-9 IN HUMAN MESANGIAL PROLIFERATIVE GLOMERULONEPHRITIS

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Matrix metalloproteinases (MMPs) are the major regulators of extracellular matrix (ECM) degradation in the glomerulus. Recent studies revealed that MMPs are involved in the development of glomerulonephritis (GN) in rat model. Furthermore, the expressions of MMPs in the human GN are implicated. In order to examine the role of MMPs in human GN, we performed immunohistochemistry with polyclonal anti-MMP-2 and MMP-9 antibodies, and gelatin zymograms of isolated five glomeruli, in various types of human renal disease. The investigated renal specimens tissues consisted of normal kidneys (N=5), IgA nephritis (N=20), Henoch-Schölein nephritis (N=4), non-IgA mesangial proliferative GN (N=9), lupus nephritis (N=6), acute poststreptococcal GN (APSGN) (N=4), and diabetic nephropathy (DN) (N=4). Immunohistochemical analysis showed that MMP-2 expression was not detected in normal controls and any types of GN. MMP-9 expression was almost negative in normal glomeruli, but increased mainly in the mesangial region corresponding to the level of glomerular cell proliferation in GN (IgA nephritis, Henoch-Schölein nephritis, non-IgA mesangial proliferative GN and lupus nephritis). Positive, but weak, expression for MMP-9 was observed in mesangial areas in DN. Additionally, double immunostaining showed that MMP-9 is colocalized in scattered neutrophils within diseased glomeruli in APSGN. Gelatin zymograms revealed that MMP-9 activity was weakly found in normal five glomeruli. Consistent with the levels of glomerular MMP-9 expression, MMP-9 activity was dramatically increased in nephritic glomeruli with IgA nephritis, lupus nephritis and DN. The gelatinolytic activity of MMP-2 was occasionally detectable in nephritic glomeruli. These findings suggest the possible role for MMP-9 in the pathophysiology of mesangial proliferation in the human mesangial proliferative GN.

M231 PERIPHERAL BLOOD AND URINARY MONONUCLEAR CELLS IN GLOMERULONEPHRITIS: ASSOCIATION WITH DISEASE ACTIVITY AND EFFECTS OF ANGOIOTENSIN CONVERTING ENZYME INHIBITION

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Glomerular and interstitial mononuclear leukocytes, especially macrophage accumulation is frequently seen in most forms of glomerular diseases. Various studies have shown that inhibition of renin-angiotensin system (RAS) resulted in reduction of mononuclear leukocyte recruitment in several renal injury models. In this study, the effect of RAS inhibition on urinary mononuclear cell numbers and characteristics were studied in patients with various glomerulonephritis (GN) Twenty-three patients (13M, 10 F, mean age 35±12) with newly diagnosed glomerular diseases were included. All patients had renal biopsy and were divided into two groups according to their renal biopsy findings as proliferative (n=13, mean proteinuria 3.15±2.31 g/day) and non-proliferative GN (n=10, mean proteinuria 4.26±5.79 g/day). CD16+ monocytes (proinflammatory monocytes) and natural killer (NK) cells in peripheral blood and the percentage of macrophages, CD16+ macrophages (activated macrophages), T and NK cells in urine were determined using flow cytometry. Twenty-one patients had enough urinary leukocytes to be analyzed. Urinary macrophage:T cell ratio of patients with proliferative GN were significantly higher when compared with non-proliferative group ($p<0.05$). Twelve patients (6 proliferative, 6 non-proliferative) received angiotensin converting enzyme (ACE) inhibitor, quinapril (20 mg/day) before institution of immunosuppressive therapy. After 2 weeks of treatment, a significant reduction in the percentage of CD16+ monocytes in peripheral blood ($p<0.05$) were reduced. Urinary macrophages also decreased after quinapril treatment ($p<0.05$).These results suggest that urinary macrophage:T cell ratio may be a disease activity marker and RAS inhibition may modulate monocyte/macrophage activation and macrophage accumulation in glomerular diseases.

M232 RENAL BIOPSY PROTOCOL FOR MONASH MEDICAL CENTRE: A SPECIALIZED APPROACH

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A percutanous renal biopsy is commonly performed to provide adequate renal tissue for examination. The adequacy of a renal biopsy specimen is determined by the amount of renal cortex present and by the type of disease being investigated[1].

This institution over a 2 year period handled 417 native renal biopsies and 124 renal transplant biopsies. All of these biopsies were investigated using light microscopy and immunohistochemical techniques. Electron microscopy was performed on all native renal biopsies.

Tissue for light microscopy is fixed in 10% neutral buffered formalin for 60-90 minutes and processed by hand through conventional methods. Many conventional fixative protocols mask, alter and destroy some antigens, therefore this limited fixation time is combined with a post-fixation step of mercuric formalin and Bouins. This enhances the staining properties otherwise lost by the preservation of antigenicity. Sections are cut at 0.5*um* (compared to 3-4*um* for non-renal tissue) and mounted on 24 labelled slides. Representative sections are stained with haematoxylin and eosin, periodic acid-Schiff, Masson trichrome, Silver methenamine-Masson trichrome and orcein-Masson trichrome stains. The cutting of thin (0.5*um*) sections is an integral part in the interpretation of a renal biopsy. The position of deposits along the glomerular basement membrane (GBM) and within the mesangium of a glomerulus is better defined, especially after staining with silver methenamine[1]. Thin serial sections can be obtained by cooling the block on dry ice.

The decision on whether to use immunoperoxidase (IP) or immunofluorescence (IF) is dependent on many technical, diagnostic and economical factors. Immunoperoxidase provides a more precise localization of the antigen than IF and provides a permanent record. However, paraffin embedding markedly decreases or sometimes abolishes reactivity of antigens. The linear deposition of IgG along the GBM in anti-GBM disease is unreliable by IP, and a definitive diagnosis can only be made by IF. The IF findings are also important to rule out other causes of crescentic glomerulonephritis[2]. Because of this, tissue is taken for IF if there is adequate tissue available. It is then snap frozen in OCT compound in liquid nitrogen and stored at a temperature below -15°C. Sections are cut at 4*um* in a cryostat and incubated with IgA, IgG, IgM, C_3, C1q, C_4, albumen and fibrinogen.

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M233 INCIDENCE OF GLOMERULOPATHIES IN THE CZECH REGISTRY OF RENAL BIOPSIES (CRRB) IN THE YEAR 1995-2001

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Since 1994 the CRRB is organised to extend the information about the epidemiology of glomerulonephritides (GN) in our region. Currently 28 Czech renal units collect basic clinical-laboratory and renal biopsy (RB) data covering almost all population of the country (10.3 mil.). We have evaluated the occurrence of primary and secondary glomerulonephritides in a period of 7 consecutive years (1995-2001).

During this period, altogether 4159 renal biopsies (RB) records were collected, 660 of them were performed by children (age < 16 years).

Primary glomerulonephritis (GN) was detected in 59.3% [33.3 per one milion of inhabitans per year (PMPY)], secondary GN in 24.7% [13.9 PMPY],tubulointersticial nephritis 4.5% [2.5 PMPY], nephrosclerosis 3.0% [1.7 PMPY], ESKD in 1.1% [0.6 PMPY]. Normal renal tissue was found in 1.3% [0.7PMPY]. In 4.7% of samples diagnosiscould not be established.
Among primary GN the most common diagnoses were: IgAN, minimal change nehrotic sy, FSGS (34.0%, 15.4%, 10.9%) [11.3, 5.2, 3.6 PMPY]. Secondary GN were divided into two main subgroups: immune mediated GN (A) and GN with dysgamaglobulinemia (B). In A the most frequent were lupus nephritis, vasculitis and Henoch-Schonlein purpura (50.7%, 26.5% and 14.3%) [3.3, 1.7, 0.9PMPY], in subgroup B: amyloidosis, multiple meyloma and mixed cryoglobulinemia (64.9%, 18.71% 7.0%) [1.4, 0.4, 0.15PMPY].
In conclusion: in comparison with the only other available national registry in Europe (Italian registry) the prevalence of major GN is similar, but children and minimal change disease are overrepresented possibility due to the different biopsy policy.

M234 EPIDEMIOLOGIC STUDY OF GLOMERULAR DISEASE -CASE SERIES REPORT

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The epidemiology of glomerulonephritis (GN) is diverse in different parts of the world. Membranous GN has been introduced as the most common cause of nephrotic syndrome in many reports and IgA nephropathy as the most frequent type of primary glomerular disease in some parts of the world. As we have little data about the epidemiology of different types of GN in our country, this study was done to delineate the frequency of different types of GN and the clinico-pathologic correlation of renal biopsy findings in our hospital.
Hospital charts from patients who had undergone renal biopsy (Bx) from Sep. 1998 to Sep. 2001 were reviewed. Demographic data, laboratory findings, pathologic diagnosis and clinical syndromes at presentation were studied. Pathologic diagnosis was based on light microscopy and immunofluorescence findings. Charts with no definite pathologic diagnosis were excluded.
Of the total 462 renal Bx cases, 267 (57.8%) patients were male and 195 (42.2%) female. Mean age at the time of renal Bx was 36.1±16.1 years (13-76 yrs). The clinical syndromes leading to decision for renal Bx were nephrotic syndrome (58.9%), nephritic syndrome (15.6%), chronic renal failure (9.7%), acute renal failure (8.9%), asymptomatic urinary abnormality (3%), rapidly progressive renal failure (1.9%) and sub-nephrotic proteinuria (1.1%). 48.2% of patients had serum creatinine> 1.4 mg/dl and 48% were hypertensive at presentation.
The most common type of primary glomerular disease was membranous GN (MGN) consisting 20.8% of renal Bx cases, followed by FSGS in 12.1%, IgA nephropathy in 11.9%, MPGN in 10.2%, minimal change disease in 8.7%, chronic GN in 5.4%, diffuse crescentic GN in 4.5%, diffuse proliferative GN in 2.4% and mesangial proliferative GN in 1.9%. The most common type of secondary glomerular disease was lupus nephritis in 9.3% of patients, followed by amyloidosis in 3.5%, hypertensive kidney in 0.9%, diabetic nephropathy and micro-angiopathy each in 0.6% and light chain nephropathy and myeloma kidney each in 0.2%. Tubulointerstitial disorders totally included 3.9% of cases, consisting of 11 cases of acute and chronic tubulointerstitial nephritis (2.4%), 6 cases of acute tubular necrosis (1.3%) and 1 case of myeloma cast nephropathy (0.2%).
In conclusion in our case series, the most common clinical syndrome leading to renal Bx was nephrotic syndrome and MGN was the most frequent type of renal biopsy finding. This is different with studies from Far East and Europe reporting IgA nephropathy as the most common primary glomerular disease and may be due to true epidemiological difference or high prevalence of nephrotic syndrome in our center, as a referral nephrology hospital. FSGS and MPGN reported to be the most frequent glomerulonephritides in Saudi Arabia and Russia respectively were in the 2nd and 3rd rank in our study. Lupus nephritis was the most common secondary glomerular disease similar to most other centers but other secondary glomerular disease especially infectious types were rare in our series.

M235 GLOMERULAR DISEASE IN CHILDREN: A REPORT FROM SOUTH ISLAMIC REPUBLIC OF IRAN

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During a 20 year period (1980-2000)percutaneous kidney bipsy was performed in 454 children with an age range of 3 months to 18 years with different presentations of glomerular diseases, including: congenital nephrotic syndrome (CNS), steroid resistant NS, steroid dependent NS, nephritic-nephrotic syndrome and isolated persistent hematuria and various systemic diseases with significant proteinuria and/or hematuria. Biopsy specimens were evaluated by light and fluorescent microscopy and in recent cases with electron microscopy too.Pathologic findings were as follows: MesPGN 94 (20.9%), FSGS 92 (20.2%), MCNS 80 (17.6%), Lupus nephritis 56 (12.3%), MPGN 49 (10.79%), RPGN 28 (6.1%), CNS 21 (4.6%), Henoch-Schonlein nephritis 16 (3.5%), IgA Nephropathy 7 (1.5%), MGN 6 (1.3%) and HUS 5 (1.1%). In conclusion MesPGN and MPGN are less common in our patients than in East and South East Asia (20.9% and 10.79% vs 36% & 21%) but FSGS is more common (20.2% vs 5.9%). Lupus nephritis 12.3%were more common than in South African children4.6%. IgA Nephropathy is much less common than other countries (North America and Japan). Although visceral leishmaniasis is endemic in this area we have not encountered significant renal involvement requiring kidney biopsy in these children.

M236 PATHOLOGICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF ADULT PATIENTS WITH RENAL DISEASE IN CENTRAL ISRAEL-A 10 YEAR RETROSPECTIVE REVIEW

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Objective: To examine the nature of biopsy diagnosed renal disease in this hospital.
Patients: All patients who had a native kidney biopsy (KB) since 1992.
Main Outcome Measures: Histopathological diagnoses and epidemiological features of patients who had KB.
Results: 192 KB's were done. 2 patients were guest workers (1 from the Philippines-with FGS (focal glomerulosclerosis), (1 from China-IgA nephropathy).The other 190 patients were Israeli Jews. 50 patients had secondary renal disease (SRD) with SLE being the most common (n=27,14.5% of all KB) and amyloidosis second (n=10, 5.4%primary=3,secondary=7). The most common primary renal disease (PRD) was IgA nephropathy (n=30,16.1%) with MCD (minimal change disease) and MN (membranous nephropathy) equal second (n=26, 14.0%) each. There were 14 cases of FGS (7.5%), with only 4 of the cases diagnosed in the last 4 years. The male:female (M:F) ratio of Lupus nephritis was 1:2.9, and in IgA nephropathy 1.7:1. MN was a predominantly male disease 3.3:1. 6 patients had ATN on KB. 5 patients with diabetes had KB, 1 had diabetic nephropathy as the predominant finding. 4 patients had interstitial nephritis. 7 patients with FMF (Familial Mediterranean Fever) were biopsied for proteinuria, 3 did not have amyloidosis.
Conclusions: IgA nephropathy was the most common PRD in this series of adults over 17 years of age. It was closely followed by MN and MCD (including IgM nephropathy). FGS at 7.5% of all biopsy diagnosed renal disease is relatively uncommon, and is not increasing in this population. This is in contrast to what is currently reported from western countries, where FGS is now more common than either MN or MCD in native kidney biopsies.

M237 GLOMERULONEPHRITIS IN OCCUPATIONAL EXPOSURE PATIENTS, A LIGHT MICROSCOPIC STUDY OF 375 AUTOPSY CASES

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Kidneys involvemennt in occupational exposured patients were sporadically reported. We had done the light microscopic review of kidneys from autopsy materials of occupational exposed patients who died during 1980-2000 at Rosai hospital for Silicosis, Japan. Among 375 patients, there were 6 cases that showed light microscopic changes compatible with membranous glomerulonephritis (2), mesangial proliferative glomerulonephritis (2), and rapidly progressive glomerulonephritis (2). Immunoperoxidase studies of Immunoglobulin G,M,A and complements C3and C1q showed no different from the primary glomeruloneprititis.
We concluded that in occupational exposured could cause kidney injury, however no specific pattern of glomerulonephritis to distinguished them from the primary glomerulonephritis. Some patients died of acute renal failure.Clinician should look for glomerular injury in occupational exposured patients who have renal impairment.

M238 FAMILIAL CLUSTERING OF DIFFERENT FORMS OF PRIMARY GLOMERULONEPHRITIS: VALTROMPIA STUDY

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Primary GN are not considered a hereditary disease. However, genetic influence on its pathogenesis is suggested by ethnic and geographic variation in the prevalence, clustering in isolated populations with limited founders, familial aggregation, and, most recently, by genetic linkage to a specific chromosomal region for IgA nephropathy. We report the results of an epidemiologic, clinical and genealogic study study performed in three small villages (Saint Colombano, Collio, Bovegno; 5.000 inhabitants) of an isolated valley (Valtrompia) of Northern Italy, where 47 patients (pts) with GN belonging to 5 large pedigrees potentially related were found.
(1) **S. Colombano:** we reconstructed a single pedigree with a common ancestor (1570) including 22 pts with various family relationships (3 parent-child pairs; 2 sib-pairs; 12 distant cousins);5 pts had IgAN; 7 mesangial GN without IgA deposits; 2 MN; 1 MPGN; 7 clinical GN.
(2) **Collio:** a single pedigree (common ancestor: 1700) contained 8 distant cousins with GN (IgAN in 2; mesangial GN without IgA deposits in 1; FSGS in 1; clinical GN in 4)
(3) **Bovegno:** 17 pts with various family relationships (3 sib-pairs; 11 distant cousins) had GN (IgAN: 4; mesangial GN without IgA deposits: 4; clinical GN: 6; FSGS: 3). The defective parish archives did not allow us to reconstruct a single pedigree for these 17 pts. Thus, 13 pts were found to belong to a fitst large pedigree (common ancestor: 1630); 2 remaining pts belonged to a second pedigree (common ancestor: 1650) containing also 10 pts members of the first pedigree; 1 pt belonged to a third pedigree (common ancestor 1700), which also contained 5 pts who were members of the first and second pedigrees from Bovegno; finally, 1 pt was found to belong to the pedigree from S. Colombano, confirming the potential relationships between the pedigrees of the three villages. In the absence of obvious environmental factors, our data confirm the role of genetic factors in the development of primary GN. The novel and exciting aspect of our study is the clustering of different forms of primary GN. The molecular genetic study will clarify this point.

M239 ACQUIRED FACTOR X DEFICIENCY IN A PATIENT WITH ESRD AND FIBRILLARY GLOMERULONEPHRITIS

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Acquired factor X deficiency is a well recognized complication of AL-Amyloidosis. In a large series of 368 patients with AL-Amyloidosis only 12 patients (3,3%) had severe bleeding complications. However, thorough examination of the clotting system revealed a 8,7% prevalence of decreased factor X levels below 50% of normal in this cohort.
We describe a 70 year-old male patient who was diagnosed to have a glomerulopathy due to nonamyloid fibrillary deposits (fibrillary glomerulonephritis) that had led to ESRD. As a result of acquired factor X deficiency he presented with massive spontaneous bleeding. Antibody formation or loss of clotting factor via urine could be ruled out. Extensive examinations, including CT-scans and immuneelectrophoresis did not show further organ involvement. Critical re-evaluation of the histology confirmed the diagnosis of fibrillary glomerulopathy. In contrast to amyloidosis it is restricted to the kidney and the fibrils do not stain with congo red. Treatment with high dose human plasma factor components (PPSB) lead to temporary increase of factor X levels and prevented further bleeding episodes. The patient could be discharged on a treatment schedule for his regular hemodialysis as an atypical hemophiliac.
This is the first description of a coincidence of acquired factor X deficiency and nonamyloid fibrillary disease. We hypothesize that binding of factor X to nonamyloid fibrils may occur in the kidney similarly as has been proposed for nonamyloid fibrils in the spleen and subendothelial space in amyloidosis.

M240 GLOMERULONEPHRITIS IN PATIENTS WITH HYDATID DISEASE (HD)

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Hydatid disease (HD) is a very common and even endemic in many countries, leading to cyst formation in several organs (liver, spleen, lung, kidney...) whereas the association HD and glomerulonephritis (GN) seems to be exceptional.
We report 24 patients with HD and histologically proven GN. They are 10 M and 14 F (mean age: 43,86 years). The first group includes 13 patients who have renal amyloidosis (RA) and the second group includes 11 patients with different types of GN: endocapillaryproliferative GN (ECPGN): 3 cases, proliferative endo and extracapillary GN: 2 cases, MCGN: 3 cases, MGN: 3 cases, FSGN: 1 case.
Among the 13 patients with AA type RA, 4 had an other possible cause to their RA (Hodgkin disease: 1, chronic broncho- pneumopathy: 2, tuberculosis: 1) whereas the 9 others had no other etiology,. One patient in the 1st group and 1 patient in the 2nd group achieved a complete remission after surgical removal of hydatid cyst.
Several parasitic diseases such as Malaria, Schistosomiasis, Filariosis and Leishmaniasis are known to be possibly complicated by GN, whereas HD although its high prevalence in many countries is very rarely associated to GN.
The rarety of such association lies the problem of the relationship between the 2 affections. However, in some reports, Hydatid antigen has been isolated in patients and animal model's glomeruli, this fact associated to the remission of GN after cyst removal may plead in favour of the responsability of HD in the occurrence of GN in such patients.

M241 ASSESSMENT OF NITRIC OXIDE (NO) AND PROSTAGLANDINS LEVELS IN DIFFERENT CLINICAL AND PATHOLOGICAL TYPES OF GLOMERULAR DISEASES

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Nitric oxide (NO) has been implicated in the induction of proteinuria in acute inflammatory glomerulonephritis. In addition,it has been shown that NO stimulates the inducible cycloxygenase enzyme (COX_2) in vitro,an observation that might explain the endothelial dysfunction and thrombotic tendency observed in proteinuric patients.
We evaluated plasma nitrite and nitrate as well as urinary nitrite levels in forty cases with different glomerular diseases. In addition,serum throm-

boxane (TXA_2) and prostacyclin I_2(PGI_2) levels were examined in these cases in comparison to twenty healthy controls. Serum cyclic guanosine 3,5 monophosphate (cGMP) levels were estimated as it was reported that NO mediates its inflammatory effect through activation of cGMP. The effect of steriod therapy on the above mentioned parameters was also evaluated.
Urinary nitrite levels were significantly increased among patients compared to controls (P<0.05),the elevation was observed in *diabetic nephropathy* as well as *proliferative glomerulonephritis* (GN) such as mesangialproliferative GN and membranoproliferative GN. However,plasma nitrite and nitrate levels and cGMP levels were similar to control levels (P>0.05). Furthermore,there was no significant correlation between plasma nitrite,nitrate and urinary nitrite levels on one hand and serum PGI_2,TXA_2 and the extent of proteinuria on the other hand. TXA_2 levels exhibited significant elevation in patients coupled with significant decrease in PGI_2 levels compared to control subjects (P<0.05). Steriod therapy was demonstrated to have no effect on all of the study parameters.
Our data indicate that NO may play a role in the pathogenesis of proliferative GN and diabetic nephropathy. The demonstration of normal levels of urinary nitrite in non proliferative GN as well as the lack of a positive correlation between urinary nitrite levels and the extent of proteinuria suggests that enhanced NO production may not be the sole cause of proteinuria in these cases. The lack of significant increase in plasma nitrite and nitrates among patients suggested that NO is produced mainly within the kidney. This is also supported by the normal levels of cGMP demonstrated in patients in this study. The significant elevation of TXA_2 levels with significant decrease in PGI_2 levels should be throughly investigated regarding the renal hemodynamics,the extent of proteinuria and the thromboembolic tendency reported in these cases.

M242 ENDOTHELIAL FUNCTION, INFLAMMATION, AND PROTEINURIA IN PATIENTS WITH PRIMARY GLOMERULONEPHRITIS

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The increased incidence of cardiovascular mortality is well established in patients with end stage renal disease (ESRD) or the nephrotic syndrome. The magnitude of the increase in risk is unlikely to be explained by traditional risk factors for cardiovascular disease alone. Recent epidemiological evidence suggests that in both essential hypertension and type II diabetes, the development of microalbuminuria is associated with a marked increase in the risk of cardiovascular mortality. Whether proteinuria directly influences endothelial function, or whether there is an intermediate pathogenetic factor, is the subject of intense investigation.
The aim of this study was to examine the relationship between endothelial dysfunction, as reflected by serum von Willebrand factor (vWF), and proteinuria in patients with primary GN. A secondary aim was to discern whether any relationship could be explained on the basis of differences in renal function, lipid profile, inflammation or blood pressure.
A cross-sectional study was undertaken in consecutive patients attending a general nephrology clinic with biopsy proven primary GN. Patients with ESRD, those on immunosuppressive drugs, or with intercurrent infective illnesses were excluded. Blood pressure, and body mass index were recorded. Routine lab assays were undertaken for serum creatinine, lipid profile, and 24 hour urinary protein (QP). Extra serum samples were stored at –80°C, to be thawed in batches for measurement of vWF and sensitive C reactive protein (sCRP).
Data were collected from 129 (86 male) patients (IgAN 39, MGN 36, FSGS 28, MCGN 10, others 8). Mean (± SD) estimated creatinine clearance was 64 (± 32) ml/min, and median (IQ range) 24 hour proteinuria was 1.1g (0.22g-2.9g). Mean vWF was 173 (± 68) IU/dl, median sCRP was 2.33 (0.83-5.68) mg/l. There was a significant correlation between vWF and QP (r^2 17%, p<0.001). When split according to QP into 4 groups there was a significant, stepwise increase in mean vWF (H=25, p<0.001). On multivariate analysis with vWF as the continuous dependent variable, QP and age were the only significant independent correlates.
In patients with primary GN endothelial function, as reflected by serum vWF, is independently correlated with total proteinuria and age. This relationship is independent of differences in renal function, inflammation, lipid profile or blood pressure. In a cross-sectional study such as this it is not possible to comment on cause effect relationships. It is interesting, however, to speculate as to whether the relationship between proteinuria and endothelial function is a direct one, or whether it is mediated by a separate factor not measured here such as insulin resistance or oxidative stress.

QP (mg/24 hours)	0-300	300-1000	1000-3000	>3000
n	36	25	38	30
vWF (IU/dl)	142 (±58)	144 (±57)	184 (±60)	220 (±72)

M243 ASSOCIATION OF RENAL HAEMODINAMICS DETERMINED BY DOPLLER ULTRASOUND WITH MORPHOLOGICAL ACTIVITY AND CHRONICITY INDICES IN PATIENTS WITH CHRONIC GLOMERULONEPHRITIS

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The aim of the study was to assess the relationship of parameters of renal blood flow with severity of immune inflammation and degree of nephrosclerosis in chronic glomerulonephritis (GN) patients. Fourteen patients with GN (10 males, 4 females, mean age 33.5±1.8) were studied. Mean serum creatinine was 1.4±0.5 mg/dl; mean proteinuria was 3.8±3.0 g/day.
The morphological activity (AI) and chronicity indices (CI) were obtained by summing of semiquantitative scores of certain histological features in renal biopsy specimen: glomerular and interstitial infiltration, mesangial, endothelial and epithelial proliferation, vasculities, immunoglobulin deposition – for AI; and glomerulosclerosis, synechia, sclerosis and hyalinosis of capillary loops and capsule, periglomerular and interstitial sclerosis, tubular atrophy, arteriolosclerosis and arteriolohyalinosis – for CI. The maximum score of AI could be 21, CI – 24. Systolic, diastolic and mean velocities (Vs, Vd, Vm), resistive and pulsatility indices (RI, PI) were estimated in renal (RA) segmental (SA), interlobar (IA) and arcuate arteries (AA) using Doppler US device (GE Logiq 400CL PRO Series).
The mean AI in the total group was 5.2±2.0, SI – 11.3±5.3. AI correlated significantly with PI in SA (R=0.71, P<0.001), in IA (R=0.54, P<0.05), in AA (R=0.92, P<0.001), and with RI in SA (R=0.57, P<0.05), in AA (R=0.93, P<0.001). Endothelial proliferation had the strongest correlation with PI and RI among the morphological activity parameters. CI significantly negatively correlated with blood flow velocities: in RA – Vd (R=-0.63, P<0.05), in SA – Vs and Vm (R=-0.70, P<0.001 and R=-0.60, P<0.05 respectively) and in IA – Vs (R=-0.57, P<0.05), Vd (R=-0.54, P<0.05), Vm (R=-0.57, P<0.05).
We conclude that nephrosclerosis was associated with lowering of renal blood flow. Active immune inflammation correlated with PI and RI elevation in intrarenal arteries, which could be connected with endothelial damage and dysfunction.

M244 URINARY MONOCYTE CHEMOTACTIC PROTEIN-1 (MCP-1), REGULATED UPON ACTIVATION NORMAL T-CELL EXPRESSED AND SECRETED CHEMOKINE (RANTES) AND TRANSFORMING GROWTH FACTOR-BETA1 (TGF-BETA1) IN CHILDREN WITH CHRONIC GLOMERULONEPHRITIS

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Increased renal expression and production of several growth factors and chemokins play an important role in the pathogenesis and progression of glomerulonephritis (gn). MCP-1, RANTES are involved in the accumulation of monocytes in the renal interstitium and the stimulation of fibroblasts/myofibroblasts leading to interstitial inflammation and fibrosis. TGF-beta1 is one of the most fibrogenic growth factors, influencing extracellular matrix metabolism. Severe studies suggest that increased urinary concentrations of cytokins may reflect an enhanced production of this polypeptide by the kidney cells. The aim of this study was to estimate urinary con-

centration of TGF-beta1, MCP-1, RANTES in children with chronic gn with regard to histopatological diagnosis and clinical course of disease. 72 children with chronic gn (34 girls, 38 boys, aged 5 to 17 years) and 20 age-matched healthy subjects were enrolled in the study. Urinary TGF-beta-1, MCP-1 and RANTES concentrations were determined by Elisa method and normalised to the creatinine (cr) concentration in urine. Results: Urinary TGF-beta-1 (33.36 ± 10.29 ng/mg cr), MCP-1 (798.25 ± 263.45 pg/mg cr) and RANTES (16.39 ± 6.27 pg/mg cr) level was significantly higher ($p<0.001$) in children with chronic gn than in healthy controls (11.44 ± 0.84 ng/mg cr, 142.84 ± 29.68 pg/mg cr and 9.89 ± 9.86 pg/mg cr respectively. The highest concentration of TGF-beta1 and RANTES was observed in children with focal segmental glomerulosclerosis. Urinary TGF-beta1 levels correlated positively with clearance of creatinine and proteinuria, but not with disease duration and arterial blood pressure. In conclusion: in chronic gn urine secretion of TGF-beta1, RANTES and MCP-1 was increased. These cytokins may contribute to progressive renal insufficiency. Urinary secretion of TGF-beta1 was associated with some factors of renal failure progression.

M245 THE EFFECT OF Th2-DEPENDENT CYTOKINE GENE EXPRESSION ON PROGRESSION OF PRIMARY GLOMERULAR DISEASE

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We have previously reported on a significant relation between glomerular expression of mRNA for proinflammatory cytokines/growth factors and progression of primary glomerulonephritis. Herein we summarize results of study evaluating the impact of this parameter on a Th2-lineage cytokine IL-10, regarded as antiinflammatory, on early clinical course of glomerular disease.
Glomerular expressions of mRNA for cytokines IL-10 and IL-6 were determined with the RT-PCR in situ semiquantitative method in diagnostic needle biopsy specimens of 28 patients with newly diagnosed primary glomerulonephritis. Subsequently, patients were observed for 24 months and their clinical parameters noted for assessment of possible correlations. Our data indicate that glomerular expression of mRNA for IL-10 is significantly inversely correlated with serum albumin concentration at 24 months follow-up (P=0.001), as well as correlates with initial proteinuria (P=0.027). Index of IL-6/IL-10 is not significantly correlated with any parameter of the study.
Despite earlier suggestions of an antiinflammatory potential of the Th2-dependent cytokine IL-10, our results indicate it's nephritogenic influence comparable to well established proinflammatory cytokine IL-6 and growth factor PDGF.

M246 PROTECTIVE SIGNIFICANCE OF INTERLEUKIN-10 IN PATHOGENESIS OF TUBULOINTERSTITIAL CHANGES IN CHILDREN WITH CHRONIC GLOMERULONEPHRITIS

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Aim: To find out predictive value of the urine level of interleukin-10 in presence of tubulointerstitial changes in children with chronic glomerulonephritis.
Materials and methods: We examined 15 children with resistent nephrotic syndrome: 5 children with significant tubulointerstitial changes, 5 – with moderate tubulointerstitial changes and 5 – with insignificant tubulointrstitial damage. In all of them the urine level of interleukine-10 was measured by immunoenzyme assay.
Results: We found significant decreasing of the urine level of interleukin-10 in children with chronic glomerulonephritis in increasing of tubulointerstitial injury (6±1,82 pg/ml in the group with significant tubulointerstitial changes against 34,72±4,4 pg/ml, $p<0,005$, in the group with moderate tubulointrstitial damage, and 50,24±17,94 pg/ml, $p<0,05$, in the group with insignificant tubulointrstitial injury). Conclusion. In clinical conditions we determinated increasing of disbalance of protective and damaging factors in children with progression of tubulointerstitial changes: decreasing of urine level of antiinflammatory interleukin-10 in increasing of the tubulointerstitial injury – marker of progression of chronic glomerulonephritis

M247 RENAL ERYTHROPOIETIN RECEPTOR EXPRESSION CORRELATES WITH THE SEVERITY OF HUMAN GLOMERULONEPHRITIS

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Erythropoietin (EPO), the principal hematopoioetic cytokine that regulates erythropoiesis by binding to its transmembrane receptor (EPOR), promotes the proliferation and differentiation of erythroid precursor cells while also inhibiting their apoptosis. Recent evidence has suggested that the biologic effects of EPO are not limited to the regulation of erythropoiesis. Expression of functional EPOR has been reported in several nonhematopoietic cell types, including vascular endothelial cells, neuronal cells, myoblasts and kidney. The renal cell types that have been shown to express EPOR include tubular epithelial cells, glomerular endothelial and mesangial cells. These findings suggest that EPO produced primarily in mature kidney may act on the behavior of renal intrinsic cells under some physiological or pathological conditions. The purpose of the present study was to evaluate clinical specimens from a series of patients with glomerulonephritis (GN) for EPOR expression and characterize the relationship between renal EPOR expression and several clinicopathological parameters.
We studied a total of 52 renal biopsies from the patients with childhood-onset GN (4 minimal change disease, 8 focal segmental glomerulosclerosis, 6 non-IgA mesangial proliferative GN, 16 IgA nephropathy, 9 lupus nephritis, 5 membranous nephropathy and 4 membranoproliferative GN). Renal EPOR expression was assessed by immunohistochemistry and in situ hybridization. Immunohistochemical expression of α-smooth muscle cell actin (α-SMA), a marker for monocytes and macrophages (CD68), and podocyte markers (synaptopodin and WT-1) were also examined.
In normal kidney and minimal change disease, immunohistochemical expression of EPOR was weakly observed in vascular endothelial cells, tubular epithelial cells and some glomerular endothelial and epithelial cells. The localization of EPOR mRNA expression was consistent with the area showing positive immunostaining. In proliferative forms of GN including IgA nephropathy and lupus nephritis, increased expression of EPOR protein and mRNA was observed in the lesions of mesangial proliferation, crescent formation, some podocytes and tubulointerstitium. Glomerular EPOR expression was occasionally dominant in podocytes especially in focal segmental glomerulosclerosis. Serial section and confocal microscopic analysis revealed that EPOR was co-expressed with α-SMA in mesangium. A marked increase in EPOR expression on podocytes was frequently associated with the ultrastractural findings indicating podocyte injury. By quantitative analysis, the glomerular expression score of EPOR was significantly correlated with the degree of proteinuria.
In conclusion, glomerular EPOR expression is markedly upregulated in proliferative forms of GN and this correlates with histologic damage, mesangial α-SMA expression, and the degree of proteinuria. This result suggests that EPO signaling may represent a novel mechanism of mesangial activation and podocyte injuries in proliferative GN.

M248 ✻ INFLUENCE OF GENETIC POLYMORPHISMS OF RENIN ANGIOTENSIN SYSTEM ON CHRONIC GLOMERULONEPHRITIS

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I/D polymorphism of angiotensin converting enzyme (ACE)-gene, M235T polymorphism of angiotensinogen (ATG)-gene and A1166C polymorphism of angiotensin II-type 1 receptor (AT_1R)-gene have been associ-

ated with cardiovascular complications. We evaluated the impact of these three mayor polymorphisms of the renin angiotensin system on chronic glomerulonephritis.
The clinical course of n= 213 patients with biopsy proven primary chronic glomerulonephritis (IgA nephropathy: n= 107, focal segmental glomerulosclerosis: n= 62, membranous glomerulonephritis: n= 44) was studied. The mean follow up was 6.3 ± 5.2 years. According to the slope of the curve of the reciprocal serum creatinine against time ($\geq$ or<-0.1 dl * mg^{-1} * $year^{-1}$) group A (slow progressors, n= 139) and group B (fast progressors, n= 74) were defined. One hundred volunteers were analysed as controls. The polymorphisms were determined by PCR amplification followed by restriction digestion with MSP-I and Dde-I for ATG-M235T and AT_1R-A1166C polymorphisms respectively.
The allele frequencies of the polymorphisms studied were similar in patients and control subjects. Age, renal function (serum creatinine and endogen creatinine clearance) and proteinuria did not differ significantly among patients with different genotypes at the time of renal biopsy. ATG-M235T polymorphism correlated with the mean arterial blood pressure values (MM: 105.5±10, MT: 110.4±11, TT: 111.7±12 mmHg, $p<0.05$) as well as with the number of antihypertensive agents taken (MM: 1.5±1.0, MT: 2.1±1.1, TT: 2.7±1.4, $p<0.001$). ACE-ID and DD genotypes were significant more frequent in group B (fast progressors: II: 12.2%, ID: 59.5%, DD: 28.4%) than in group A (slow progressors: II: 23.6%, ID: 53.2%, DD: 20.1%, $p<0.05$). Combined analysis of ACE-I/D and AGT-M235T polymorphisms detected an interaction on affecting progression: patients carrying three or four ACE-D or AGT-T alleles (ID/TT, DD/MT and DD/TT genotypes, n=65) were found to be significant more frequent in group B (fast progressors, $p<0.01$). Patients with the ID/TT, DD/MT or DD/TT genotypes showed a worse outcome in the ten years Kaplan-Meier analysis of the kidney survival ($p<0.01$). No association between AT_1R-A1166C polymorphism and any of the parameters studied was observed.
Our results suggest that ACE-I/D polymorphism is an useful marker of progression in chronic glomerulonephritis, especially when analysed in combination with angiotensinogen M235T polymorphism. ATG-M235T polymorphism further influenced the severity of hypertension. The present study showed evidence for genetically determinated subgroups having impact on the progression of chronic glomerulonephritis.

M249 AMOUNT OF URINARY TRANSFERRIN IS A PREDICTOR OF RENAL DYSFUNCTION IN PATIENTS WITH PRIMARY GLOMERULONEPHRITIS

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It is reported that iron deficiency prevents the development of tubulointerstitial disease and the deterioration of renal function in experimental glomerulonephritis (GN). It is postulated that tubulointerstitial injury is induced by iron dissociated from transferrin (Tf) in the acidic environment of tubular fluid. Therefore, we studied the correlation between urinary Tf and the deterioration of renal function in patients with GN.
Subjects were ninety outpatients (20-80 yo, M:F=1:1) with primary GN followed at the Division of Nephrology in our hospital. All the patients were asymptomatic. Evaluations of serum creatinine, blood urea nitrogen and creatinine clearance (Ccr) were made every six months for up to 3 years. Urinary protein, α1-microglobulin, N-acetyl-β-D-glucosaminidase (NAG) and Tf were also measured.
There was not a significant correlation between urinary protein excretion and Ccr. But, urinary Tf/Cr showed an exponential increase as urinary protein excretion increased and showed a significant negative correlation with Ccr. Patients with urinary Tf excretion of over 3,000μg/gCr showed a gradual and steady decrease of Ccr. On the other hand, in patients with Tf excretion less than that, Ccr was stabilized throughout the follow-up period. Urinary Tf/Cr showed a significant positive correlation with urinary NAG and α1-microglobulin.
These results suggest that the ratio of urinary Tf to creatinine is a useful predictor of end-stage renal disease in patients with chronic proteinuric nephropathies.

M250 THE VVANNTT STUDY: VERAPAMIL VERSUS AMLODIPINE IN NONDIABETIC NEPHROPATHIES TREATED WITH TRANDOLAPRIL

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The role of calcium channel blockers (CCB) in patients with proteinuria (P) is controversial. The primary aim of this study was to test whether the combination of the non-dihydropyridine CCB verapamil (V) or the dihydropyridine CCB amlodipine (A) with the ACE-I trandolapril (T) reduced P more than T alone in patients with nondiabetic proteinuric nephropathies. The secondary aims were to evaluate the effects of these treatments on the selectivity of P (SI, as Clearance IgG/Clearance Albumin) and check their safety. Sixty-nine patients with nondiabetic nephropathies were evaluated (49 M, 20 F, age 54± 2 y, Screat 1.9± 0.9 mg/dL, baseline blood pressure (BP) 136± 90 mmHg). After a 1 month wash-out period from ACE-I or ARB, patients were given T 2 mg in open conditions for 1 month; then were randomly assigned to receive for 8 months, in a double blind fashion, T2 mg+V180 mg (n=33), or T2 mg+A5 mg (n=36).
P diminished significantly during monotherapy with T in the whole group of patients from (mean± SEM) 3078± 244 to 2537± 204 mg/24h ($p = 0.005$). In the randomized phase there was a further slight reduction of P in both groups, without significant differencies within and between treatments (T+V from 2335± 233 to 2124± 247 mg/24h; T+A from 2715± 325 to 2671± 469 mg/24 h). SI was slightly and not significantly reduced in patients treated with T+V, from (median and interquartile range) 0.20 (0.13) to 0.16 (0.15) (p = n.s.), while it significantly increased from 0.20 (0.14) to 0.30 (0.14), ($p = 0.0001$) in patients treated with T+A. After randomization Screat increased from 1.8± 0.2 to 1.9± 0.2 mg/dL in the T± V group (p=n.s) and from 2.2± 0.2 to 2.5± 0.2 mg/dL in the T± A group (p=0.001). In the whole group of patients, the modifications of SI and Screat from randomization to the end of the study were significantly directly correlated ($r = 0.45$, $p = 0.001$). BP at the end of the study was 127± 3/83± 1 mmHg in the T+V group and 125± 3/83± 1 in the T+A group (p=n.s.). The number of patients reporting adverse effects was significantly higher in the T+A than in T+V group (63.8% vs 33.3%. $p = 0.016$).
In patients with nondiabetic proteinuric nephropathies treated with the ACE-I trandolapril, the association of verapamil or amlodipine does not significantly increase its antiproteinuric effect. The possibility that amlodipine exerts an adverse effect of the selectivity of P and on renal function deserves further studies. The association of T+V was better tolerated than the association T+A.

M251 RENAL HAEMODYNAMIC RESPONSE TO ACE INHIBITOR IN CHRONIC GLOMERULONEPHRITIS DETERMINED BY COLOR DOPPLER SONOGRAPHY IN ACUTE CAPTOPRIL TEST

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The disorders of renal haemodynamics associated with intrarenal renin-angiotensin system overactivity play an important role in progression of nephrosclerosis. The aim of the present study was to evaluate the blood flow in renal and intrarenal arteries by Doppler ultrasound examination in patients with chronic glomerulonephritis (GN) with normal or deteriorated renal function and its response to renin-angiotensin system blocade by captopril (C).
26 patients with chronic glomerulonephritis (GN) (21 males, 5 females, mean age 37.1±15.0) were studied. 16 pts had normal renal function (mean serum creatinine (Cr) 1.0±0.2 mg/dl) and 10 pts had mild CRF (mean Cr 2.0±0.6 mg/dl). Systolic, diastolic and mean velocities (Vs, Vd, Vmean), resistive and pulsatility indices (PI, RI) were estimated in renal (RA) and intrarenal arteries: segmental (SA), interlobar (IA), arcuate (AA) before and after administration of 50 mg C using Doppler ultrasound device (GE Logiq 400CL PRO Series).

The following data (Mean ± SD) were received (Table 1).

Renal haemodynamic response to acute captopril test

		Vs	Vd	Vmean	PI	RI
RA	before C	97.5±23.5	36.7±8.7	52.0±12.9	1.16±0.19	0.64±0.05
	after C	115.5±39.1**	42.8±3.7**	62.9±18.3**	1.13±0.29	0.63±0.05
SA	before C	64.2±15.8	27.8±7.7	40.3±9.2	0.94±0.14	0.58±0.05
	after C	74.8±24.8**	32.6±11.4**	46.8±15.3**	0.93±0.19	0.50±0.06
IA	before C	38.1±9.9	17.1±4.7	24.4±6.3	0.90±0.16	0.56±0.06
	after C	44.6±13.5**	21.1±6.9**	28.4±9.1**	1.18±1.6	0.56±0.07
AA	before C	29.7±8.1	15.2±4.2	20.2±5.3	0.75±0.18	0.50±0.09
	after C	33.9±7.7**	16.7±3.4*	22.1±4.7*	0.84±0.13	0.53±0.05

* - P<0.05; ** - P<0.01 before/after C intake by Wilcoxon test

In total the increase of blood flow velocities without changes of PI and RI in all investigated arteries was observed after C intake. Significant blood flow velocities rise was found both in pts with normal and deteriorated renal function. Blood flow velocities increase correlated with young age (P<0.01) and systolic blood pressure reduction (P<0.05). 5 pts which yilded no blood velocities increase in C test had significantly older age (in 4 it was over 52) but they did not differ from the other pts by renal function. We conclude that renin-angiotensin system blocade by C improved renal haemodynamics in GN including pts with mild CRF. The older age was associated with risk of poor haemodynamic response to C.

M252 HIGH DOSAGES OF ARA 1 AND ACE INHIBITORS IN RENAL PROTECTION

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Angiotenzin have much higher kidney tissue concentrations than in blood. We assumed that retardation of kidney function loss need more higher blocking of this substrate. The aim of the study was to estimate high dosages of ARA 1 and ACE inhibitors in renoprotection. During 5 years follow-up we conducted the randomized prospective multi-centre study of usage combined therapy with ARA 1 and ACE inhibitors in 126 pts aged 4-62 with glomerulonephritis (GN). Impaired renal function in the disease onset was proved in 23 pts. The dosage of losartan was up to 3 mg/kg or irbesartan up to 8 mg/kg or telmisartan up to 4 mg/kg in combination with enalapril up to 2 mg/kg or fosinopril up to 1 mg/kg or diltiazem up to 10 mg/kg. A criterion for dosage individualization was hypotension. 131 pts with GN on a traditional dose of ACE inhibitors and ARA 1 was enrolled in the study as control group. The data obtained demonstrated rapid effect in decrease BP. In 3-6 months period of combination therapy the reducing of proteinuria from 2,1±0,3 g/l to 0,3±0,2 g/l was noted. 4-5 years follow-up of ACE inhibitors + ARA 1 led to improvement renal function in 9 pts (39%) and its full normalization in 5 (21%) pts. No side effects were revealed except for reversible serum creatinine raise during starting the therapy. Comparing with the traditional usage of ACE inhibitors or ARA 1 showed less positive effect in proteinuria reduction (2,0±0,3 g/l to 0,9±0,1 g/l) and no significant improvement of renal function. We confirm 3 possible dosages of ARA 1 and ACE inhibitors. First (traditional doses) – hypotensive effect. Second (middle doses) – antiproteinuric effect, third – high doses – renoprotective effect with possible improving of impaired renal function. We also proved the safety of high doses ACE inhibitors and ARA 1 usage.

M253 CYCLOOXYGENASE (COX)-2 SELECTIVE INHIBITORS + ACE INHIBITORS: A NEW PROTOCOL IN THE TREATMENT OF HEAVY PROTEINURIA

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Non steroidal anti-inflammatory drugs (NSAIDs) are surely useful in the treatment of proteinuria, but these agents may cause deleterious effects on kidney function as maintenance of renal perfusion and glomerular filtration; moreover often they induce gastrointestinal toxicity. Recent studies have demonstrated that COX-2 is constutively expressed in renal tissues; this isoform is intimately involved in prostaglandin-dependent renal omeostatic processes. Drugs that selectively inhibit COX-2 might therefore be expected to produce effects on renal function similar to non selective NSAIDs.

Aim of our study was to observe the efficacy of a COX-2 selective inhibitor (rofecoxib) associated with an ACE inhibitor (ramipril) on proteinuria and on renal function of chronic glomerular diseases; we compared this group with another omogeneous pool of patients previously treated with ramipril and a NSAID (meclovanate) with good results on proteinuria, but 36.6% of side effects and 3 patients in drop-out.

Eighteen patients affected with primitive chronic glomerulonephritis were treated with rofecoxib (25 mg/day) + ramipril (10 mg/day) for six months. All of them were not responsive to single treatment with ramipril. We observed daily proteinuria, renal function as corrected creatinine clearance, mean arterial pressure (MAP), serum creatinine and electrolytes. During all the period of our observation no changes were in diuretic and immunosuppressive therapy.

Our results show a significative reduction of proteinuria from 4.9 to 1.1 gr/day (p<0.002); no modification in MAP, serum electrolytes. Creatinine clearance moved from 95.3 to 97.8 ml/min, with no significative statistical modification. No patient showed gastrointestinal side effects induced from drugs.

In conclusion, we propose this combined therapy (rofecoxib + ramipril) in the treatment of chronic proteinuria in patients resistant to single therapy (ramipril), without important side effects. We considerer this protocol a better and safer treatment in front of NSAID + ACE inhibitor.

M254 CYCLOSPORINE A (NEORAL) C2 MONITORING IN THE TREATMENT OF SEVERE NEPHROPATHIES: A PILOT STUDY

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Cyclosporine A (CyA) is used as an essential part of a pathogenic treatment for some immune glomerulopathies, with concentration controlled dosage for optimal therapeutic management. Neoral C_2 monitoring is superior to C_0 monitoring in renal and liver transplant patients. The aim of the present study was to compare the monitoring of C_2 against C_0 for the Neoral formulation of CyA in 10 patients with severe course of immune and autoimmune glomerulonephritis. They were also receiving corticosteroids and cyclophosphamide in conventional and pulse doses. Patients were followed for 3 to 12 months. Whole blood CyA levels were measured 5 minutes before and 2 hours post dose of Neoral at the steady state and drug levels were checked more than one, so 33 pairs of C_2 and C_0 concentrations were included in the study. Results as mean±SD (min-max) were as follows: Neoral daily dose 2.34±0.64 mg/kg (1.2 to 3 mg/kg); C_0 level 93.9±90.8 μg/L (25 to 230 μg/L); C_2 754.8±324.7 μg/L (362 to 1500 μg/L). Individual concentrations were rated as therapeutic, low or high, applying a target range of 75-150 μg/L and 600-1200 μg/L for C_0 and C_2 respectively. Among the 13 low C_0 concentrations observed, 8 were paired with optimal C_2 levels; 4 of the 17 "optimal" C_0 levels were paired with low C_2 concentrations; and 4 of the 11 low C_2 levels were paired with "optimal" C_0 values. There were not significant differences in serum creatinine, creatinine clearance, cholesterol, serum albumin and blood pressure at the beginning and at the end of the study. Proteinuria and episodes of hematuria were reduced significantly. No adverse effects were established during Neoral therapy. We conclude that C_2 monitoring of Neoral might have a place in the effort to optimise the therapy and avoid nephrotoxicity in patients with immune nephropathies.

M255 MYCOPHENOLATE MOFETIL TREATMENT IN PATIENTS WITH GLOMERULONEPHRITIS THAT IS REFRACTORY TO CONVENTIONAL IMMUNOSUPPRESSIVE THERAPY

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The success rate of different treatment protocols for primary glomerulonephritis (PGN) is 60-70%. Recently, it has been suggested that in some patients who are unresponsive to standard treatments, the pro-drug of mycophenolate –mycophenolate mofetil- can be used. In our study, the results of 20 patients suffering from PGN (7 membranous nephropathy, 6 membranoproliferative GN, 3 focal segmentary glomerulosclerosis, 2 IgA nephropathy, 1 proliferative GN and 1 mesengioproliferative GN) are investigated. The patients that were given MMF for 2-13 months (mean 7.35 months) were followed-up for 8.95 months on the average and given MMF 1-2 g/day. In the patients that had proliferative lesion (membranoproliferative GN, IgA nephropathy, proliferative and mesengioproliferative GN) in histopathological examination the complete response rate was found 70% whereas in the patients that did not have proliferative lesion, it was found 30%. Apart from this, in 3 patients the steroid sparing effect of MMF was observed while no serious side effect was noted. Renal function was stable in the follow-up period. Significant decreases in proteinuria had been seen beginning from the first month of the treatment. Serum protein and albumin levels were significantly higher in 1, 3, 6 and 9 months than the basal values. On the other hand serum lipids decreased significantly. The changes in the serum cholesterol, albumin and proteinuria are shown in the table.

The changes in the serum cholesterol and albumin levels and proteinuria

Months	Mean proteinuria (g/day)	Mean serum cholesterol levels (mg/dl)	Mean serum albumin levels (g/dl)
Basal	6.26	304	2.62
1. month	3.06*	248*	3.35*
3. month	2.63*	210*	3.30*
6.month	2.31*	205*	3.59*
9.month	2.30*	188*	3.60*
12.month	0.91*	-	-

*p<0.001, versus basal values

In conclusion, MMF is a well tolerable new immunosuppressive drug that enables steroid reduction and can be effective in the treatment of PGNs in which proliferative lesion is prominent.

M256 COMBINATION OF TACROLIMUS AND LOW DOSE METHYLPREDNISOLONE IN THE TREATMENT OF RESISTANT PRIMARY GLOMERULONEPHRITIS (PGN)

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The approach to the PGN resistant to conventional immunosuppressive treatment is controversial. Tacrolimus could be an alternative in the treatment of resistant cases although evidence is limited to small groups of patients with focal segmental glomerulonephritis. The aim of our study was to test the effectiveness of tacrolimus in the treatment of PGN resistant to conventional immunosuppressive treatment. 10 patients (6M:4F; mean age 40,5 ± 11 years) with PGN (2 membranopreliferative GN typeI, 2 membranopreliferative GN type II, 3 membraneous GN, 1 focal segmental GN) resistant to conventional immunosuppressive treatment were included in the study. They were started on tacrolimus 0,05mg/d aiming a trough level of 5-7ng/ml and methylprednisolone 4mg/d. After a mean follow-up of 7,9 ±5,13 months (range 3-16m) pre and post treatment serum creatinine, 24 hour urinary protein excretion, urine sediment findings were compared. The mean creatinine levels decreased from 1,67 ± 0,65mg/dL to 1,23 ± 0,34 mg/dL ($p<0,01$), the mean 24 h urinary protein excretion decreased from 2,06 ± 2,22 g/day to 0,74 ± 1,31 g/day ($p<0,02$). The greatest decrease in 24 h urinary protein excretion was observed on the fourth week of tacrolimus treatment while no simultaneous significant improvement in renal function was observed. Active urinary sediment improved in 4 of the five patients at the third month. Tremor was observed in 2 patients and de novo diabetes mellitus developed in one patient easily regulated with oral antidiabetics. No evidence of nephrotoxicity was observed in any one of the patients. In conclusion, combination of tacrolimus with low dose methylprednisolone may be a therapeutic alternative in PGN resistant to other immunosuppressive treatment. These preliminary results suggest that larger scale controlled studies are needed to investigate the efficcacy and tolerability of tacrolimus combined with low dose methylprednisolone in the treatment of resistant PGN.

B3 Hypertension: clinical

M257 ACTIVITY OF SELECTED SYSTEMS OF SODIUM TRANSPORT TROUGH ERYTHROCYTE CELLULAR MEMBRANE IN PATIENTS WITH ESSENTIAL AND SECONDARY HYPERTENSION

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High level of intracellular sodium is very significant in pathogenesis of essential hypertension (EH). Incresed intracellular sodium causes the rise of tension of smooth muscle cells in blood vessels and enchances perimeter resistance. Sodium potassium pump (ATP- Na^+/K^+), $Na^+/K^+/Cl^-$ co-transport, and Na^+/Li^+ exchange are the main systems responsible for transmembrane sodium transport.The aim of the study was to investigate if the rise of activity of sodium transport systems mentioned above can be the reason of hypertension (H) in patients with chronic renal failure (CRF). We evaluated activity of ATP-Na^+/K^+; $Na^+/K^+/CL^-$ (co-Na^+/K^+); Na^+/Li^+ exchanger (ex-Na^+/Li^+), free Na^+ and K^+ outflow (Na^+,K^+-outflow). We examined 19 persons with CRF and hypertension (cause of CRF other than EH), 18 persons EH, and 22 healthy persons (C). Patients with CRF were in pre-dialysis period. Hypertension was diagnosed according to WHO indications. Results of statistical analysis with U-Mann-Whitney test are presented in

Activity of sodium transmembrane transport systems in EH patients, CRF+H patients and C

	EH/CRF+H	EH/C	CRF+H/C
ATP-Na/K	NS	p<0,05	p<0,01
co-Na/K	NS	NS	NS
Na-outflow	NS	NS	NS
K-outflow	NS	p<0,05	NS
ex-Na/Li	NS	p<0,01	p<0,001

.Higher activity of sodium transport system may be the reason of hypertension development in patients with essentail and secondary hypertension.

M258 LACK OF MUTATIONS IN C-TERMINI OF SUBUNITS OF THE EPITHELIAL SODIUM CHANNEL IN 15-YEARS OLD GIRL WITH PHENOTYPE OF LIDDLE'S SYNDROME

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Liddle syndrome is an autosomal dominant form of arterial hypertension, resulting from mutations in the cytoplasmic C-terminus of either the beta