

**Diplomarbeit**

**The Role of Cystatin C in Renal Impairment and Disease Activity in Lupus Nephritis**

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## **Preface**

When I began to study medicine in 2002, I was driven by the idealistic thought of searching for answers. Soon I found out that it's not the answers which are the major focus but it's the questions that motivate to study a whole lot of fat books and the better you know them, the more questions emerge. This may appear a bit masochistic because it implicates that there is no end or no goal in this target but at the end of the day, it is a matter of attitude whether or not a person is ready to face the fact that the ultimate truth is an illusion and it is not "somewhere out there".

The fascination for Rheumatology also is a fascination of questions and nurturing this condition was the major task of this diploma thesis.

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## **Abstract**

**Background:** Lupus Nephritis (LN) is a severe and outcome-defining complication in Systemic Lupus Erythematosus (SLE) and until present no marker has been found which reliably detects LN early in the course of disease. Cystatin C (CysC) is described as a marker independent upon inflammation and drugs which reliably estimates the glomerular filtration rate (GFR) **Objective:** To assess the role of Cystatin C as compared to Creatinine Clearance, MDRD and serum Creatinine regarding possible influences. **Materials and Methods:** In two groups (SLE-only, n=8 versus SLE with Lupus Nephritis, n= 16) correlation analysis was conducted to determine the influences of inflammation, body composition and thyroid function upon renal parameters. **Results:** A deliberate difference in the correlation analysis regarding the renal parameters in the two cohorts was detected. The SLE-only cohort showed statistically significant lower absolute lymphocyte count and one subject presented with severe hyperthyroidism. Both factors influenced serum Cystatin C level to a great extent. **Discussion:** This is the first study to describe lymphopenia as a possible factor influencing serum Cystatin C value and additionally adds another case of inappropriate CysC level in hyperthyroid patients to current discussion.

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## **Abbreviations**

24hCr<sub>cl</sub> - 24 hour creatinine clearance

APL - Antiphospholipid syndrome

BMI - Body Mass Index

CLE - Cutaneous lupus erythematosus

CRP - C-reactive protein

CysC - Cystatin C

ELISA - Enzyme linked immuno sorbent array

GFR - Glomerular Filtration Rate

kD - Kilo Dalton

LN - Lupus Nephritis

MDRD - Modification of Diet in Renal Diseases

MMF - Mycophenolate-Mofetil

NEU - Absolute neutrophile count

NPSLE - Neuropsychiatric lupus erythematosus

SCLE - Subacute cutaneous lupus erythematosus

SLE - Systemic Lupus Erythematosus

WBC - White blood count

# **The Role of Cystatin C in Renal Impairment and Disease Activity in Lupus Nephritis**

(Cand. Med. Martin Steinkogler)

## **1. Introduction**

### **1.1. General aspects of Systemic Lupus Erythematosus<sup>1;2</sup>**

#### **1.1.1. Epidemiology**

Lupus is a multisystemic autoimmune disease of unknown origin. In Europe and North America, a prevalence of 50 per 100.000 and an incidence of 5-10 per 100.000 per year are assumed. As seen in other autoimmune diseases too, SLE prefers the female gender with a female to male ratio of 10 to 1. The occurrence of the disease heavily varies with ethnicity. People of African-American, Hispanic or Asian origin show a higher incidence and prevalence than Caucasians.

#### **1.1.2. Etiology and Pathophysiology**

The major characteristic of Systemic Lupus Erythematosus is the diversity in the clinical presentation and the course of the disease which is reflected by the multitude of alterations in many different pathophysiological pathways. Therefore, Lupus is best regarded as a syndrome. Significant alterations can be seen in the predisposing genetic constitution as well as in the regulation and effects of the humoral and cellular components of the immune system. Besides these endogenous factors, additional exogenous factors play a pivotal role in the genesis of this disease. Today, the common assumption is that only an interaction of endogenous genetic predisposition reflected by susceptibility genes and insufficient protective genes, and exogenous factors, i.e. environmental influences can cause this disease.

Susceptibility genes can be found for instance in the Major Histocompatibility Complex. Homozygous deficiency of complement components like C2, C1q, C1r, C-INH or C4 is rare but most people suffering from these deficiencies exhibit SLE or Lupus-like syndromes. The risk of originating Systemic Lupus Erythematosus rises alone if HLA-DR2 and HLA-DR3 are exhibited. From some genetic constellations even certain symptoms can be predicted. For example, the extended haplotype HLA-B8/DR3/DQw2/C4AQO is associated with disease onset after thirty-five years and the formation of anti-Ro, whereas the haplotype HLA-B7/Cw7/DR2/DQw1 is accompanied by disease onset before the age of

twenty-five, nephritis and low levels of TNF- $\alpha$ . Other important genetic alterations which go along with an increased risk for SLE can be found in TNF- $\alpha$  region or Fc-receptors regions for immunoglobulins, e.g. Fc $\gamma$ IIIR.

A hallmark of Lupus is the formation antibodies and immune complexes. The majority of pathogenic Lupus antibodies show an IgG isotype with a high avidity for self antigens and a high specificity for their epitopes. The preferred targets of the auto-antibodies derive from the cell nucleus. These antinuclear antibodies (ANA) are aimed at double stranded DNA, single stranded DNA, histone proteins, etc. In order to confront the immune system with these intranuclear components they have to be either processed to the surface membrane or they have to be somehow released into the serum, e.g. by necrosis or apoptosis. Under normal conditions, the time of maintenance for immune complexes in the serum is short which suggests an inhibited clearance of these substances by the mononuclear phagocytic cell system. The usual suspects for DNA release are UV-light, infection and drugs. Exposure to UV-light goes along with several consequences which can contribute to antibody formation: Firstly, nuclear particles and proteins are moved to the cell surface. Secondly, the cell membrane can be flipped upside down so that antigenic parts of phospholipids are exposed to the immune system. Thirdly, intracellular proteins can be modified in a way which makes them susceptible to immune cells and antibodies.

Drugs and infections might also be able to contribute to the onset of autoimmunity. A possible way for infective agents to trigger autoimmunity is the concept of molecular mimicry. It claims a similarity between bacterial or viral epitopes and autoantigens which leads to the formation of cross reactive antibodies. Lupus patients show a deliberate increase in the serum load of Epstein-Barr Virus and moreover, its epitope EBNA-1 shows a stunning resemblance to a polypeptide chain expressed on the Smith antigen SmB'/B<sup>3</sup>.

Drug-induced lupus is different from "real" SLE. The symptoms of this disease are quite the same as in SLE but in contrary, they usually disappear within weeks of discontinuation of the drug.

Recently, there has been a special focus on the role of Toll-like receptors (TLR) in the evolution of SLE. Toll-like receptors recognize bacterial and viral antigens as well as endogenous structures including heat shock proteins and molecules of the extracellular matrix<sup>4</sup>. Once activated, the TLRs play a critical role in the regulation of immune response by bridging innate and adaptive immune system. They activate dendritic cells, prolong B-cell survival and stimulate various cells in order to produce proinflammatory cytokines. Impor-

tantly, T-cell independent autoantibody production can be promoted by crosslinks between the B-cell receptor and TLR-9 bearing chromatin containing immune complexes.

Finally, it has to be noted that this chapter gives nothing but a small overview on the pathogenetic frameworks and interactions which lead to Systemic Lupus Erythematosus. T- and B-cells are missing but their importance is described below in the section on kidney damage in SLE.

A lot of factors have been described which do or can contribute to establish this disease and with the better understanding of the pathophysiological implications of genetics, of the immune system and of the environmental factors, better approaches to the treatment of this disease can be accomplished.

### **1.1.3. Diagnosis and clinical manifestations<sup>5</sup>**

The diversity of factors which are involved in the pathogenesis of Lupus is also reflected in the diversity of its clinical manifestations. Determining whether or not a patient suffers from SLE is a difficult task and using the criteria of the American College of Rheumatology (ACR) provides help. Eleven features which can be seen in the course of disease are listed and the presence of four of them indicates Lupus.

1. Malar rash
2. Discoid rash
3. Photosensitivity
4. Oral or nasal ulcers
5. Non erosive arthritis of two or more joints
6. Serositis (Pericarditis/ Pleuritis)
7. Renal involvement (Proteinuria with  $>0.5\text{g}/\text{die}$  or presence of casts)
8. CNS involvement
9. Coombs positive haemolytic anaemia, thrombocytopenia, leucopenia
10. Anti-ds-DNA-Antibodies, Anti-Sm-Antibodies, Antibodies against phospholipids
11. Antinuclear Antibodies (ANA)

The fact that four of these eleven criteria included are skin lesions emphasizes the critical role of this organ and oftentimes, they are the only manifestation of Lupus and the ACR criteria can not be met as demanded. For these situations, the terms “cutaneous lupus erythematosus” (CLE) and “subacute cutaneous lupus Erythematosus” (SCLE) have been es-

established. This implicates that Lupus Erythematosus represents a group of diseases more than a single disease. Regarding the skin, more lesions than are mentioned in the ACR criteria can be found. Hair thinning and alopecia on the scalp can be seen regularly as well as Raynaud's syndrome while Osler nodes or Janeway lesions on the palm or the planta pedis are found more infrequently.

In Systemic Lupus Erythematosus, pain of the musculoskeletal system is the symptom which can be seen the most. Arthritis is typically non-erosive and shows a distribution comparable to that of Rheumatoid Arthritis. Very often, patients complain about migrating arthralgia during a period of 24-48 hours. Mutilations appear very rarely and tenderness and mild swelling of the synovia often are the only signs of joint involvement. Symptoms which are regularly featured in musculoskeletal affection are fibromyalgia, tenosynovitis and bursitis while myositis is seen rarely.

Regarding the outcome, involvement of the kidneys and the nervous system are crucial. In 1999, the American College of Rheumatology published a standardized nomenclature for nineteen features which were subsumed under the term Neuropsychiatric Systemic Lupus Erythematosus (NPSLE). This expression reflects that both central and peripheral nervous system can be affected and thus, symptoms may be neurological or psychiatric and range from headache, aseptic meningitis, chorea, seizures, mononeuropathy, Guillain-Barré syndrome to acute confusional state, psychosis, mood disorder, etc<sup>6</sup>. Manifestations may be either directly linked to Lupus itself e.g. cerebral vasculitis or stroke as a consequence of antiphospholipid syndrome (primary NPSLE) or to the complications of disease or treatment (secondary NPSLE)<sup>7</sup>. Affection of the kidneys is described below separately.

Heart and lung can also be afflicted in multiple ways in the course of SLE. Every single structure in both organs can serve as a target of damage. Lung involvement can show interstitial fibrosis, pleuritis, embolism, lupus pneumonitis or even capillary leak. Heart affection can present as lesions of the valves (Libman-Sacks lesions), pericardial effusions or myocarditis. It is important to note that SLE appears to be a risk factor of coronary heart disease<sup>8</sup>.

Many other conditions are associated with SLE but only the antiphospholipid syndrome and fatigue should be briefly mentioned here.

Antiphospholipid syndrome (APL) occurs in about 50-60 percent in Lupus and is associated with venous and arterial thrombembolism, thrombopenia and recurrent spontaneous abortion. If more than three organ systems are involved, the term "catastrophic APL" is

used. It is caused by antibodies against cardiolipin and its diagnosis bases on the search for Lupus-Anticoagulans or ELISA for Anti-Cardiolipin-Antibodies. Treatment options range from oral anticoagulation or acetyl-salicylate acid for thrombosis or stroke, to steroids and immunosuppressive medication for thrombocytopenia or low-dose salicylate acid and low-dose heparin in case of recurrent spontaneous abortion.

Although it gets hardly any attention from the physicians, fatigue is one of the biggest problems for SLE patients themselves. The amount of studies existing on this subject is limited, but it apparently gets discussed more in recent literature. A major problem arises from the fact that fatigue does not correlate with disease activity and therefore a patient can suffer despite a seemingly good health condition. Standardized measurement is available in terms of the Fatigue Severity Score (FSS). A systematic review in recent literature showed that fatigue does not correlate with depression, poor quality of sleep and each subscale of the Short-Form-36 questionnaire<sup>9</sup>. Further problems are confounding factors such as depression which can be mistakenly mixed up with fatigue but importantly, the question on how to treat fatigue remains to be evaluated.

## **1.2. Renal Involvement in Systemic Lupus Erythematosus**

### **1.2.1 Epidemiology**

During the course of disease, thirty to fifty percent of all patients exhibit renal damage. The multicenter driven PROFILE study included 1.008 SLE patients from who 438 presented with renal damage. The investigation revealed African American or Texan Hispanic origin as risk factors for Lupus Nephritis as compared to Puerto Rico Hispanic or White Americans ancestry. Furthermore, nephritis patients were of lower socioeconomic state and showed a statistically significant risk for being unemployed. Male gender, duration of disease and the number of ACR criteria met at cohort entry were also associated with an increased risk of developing Lupus Nephritis<sup>10</sup>.

### **1.2.2. Mechanisms of Renal Damage in Lupus Nephritis<sup>11</sup>**

#### ***1.2.2.1. Non-inflammatory Renal Damage: Membranous Nephropathy***

As mentioned below, WHO class V Lupus Nephritis is not necessarily associated with signs of inflammation seen in light or electron microscopy. The major targets of disruption are the visceral epithelial cells, called podocytes. They are characterized by their interdigitating foot processes which leave a small filtration slit bridged by a slit membrane. These

diaphragms are attached to the cytoskeleton of the podocytes via linker proteins. Disturbance of these linker proteins or of the diaphragms reveals the great importance of the podocytes by causing massive protein loss which usually ends up in a nephrotic syndrome. Podocyte injury leads to effacement of the foot processes and consecutively, to apoptosis. Result of this cell death is the denudement of the glomerular basement membrane which is to be held responsible for sclerosis and ongoing renal damage.

As described above, a hallmark of Lupus is autoantigen production and formation of immune complexes. In membranous nephropathy, but also in inflammatory proliferative nephritis, deposits of immune complexes and complement factors in the glomerulum are typical. It is still unclear, if these are derived from in situ formation or by circulating complexes. Damage of these deposits is extensively linked to the membrane attack complex C5b-9 which is thought to be the principal mediator of altered glomerular barrier function. Studies showed that genetic depletion of terminal complement factors greatly reduces renal damage. C5b-9 causes formation of the cell toxic hydrogen peroxide and induces the production of cytokines which lead to an extensive formation of extracellular matrix. The major involvement of the complement system explains hypocomplementaemia which is a common feature in SLE.

#### 1.2.2.2 Role of T- and B-cells in Lupus Nephritis<sup>12</sup>

To promote lymphocyte activation, two different signals are needed in both B- and T-cells. The first signal for T-lymphocyte activation is obligatorily bound to antigen presentation together with MHC on antigen presenting cells (APC), while B-cells can receive their primary stimulus by soluble antigens, e.g. free virus particles in the serum. In the T-cell receptors/MHC complex, this first signal leads to an upregulation of CD40/L which increases to the expression of the B7 molecule. The second signal and the formation of a B7/CD28 complex leads to IL-2 secretion. This cytokine promotes a massive clonal expansion of the T-cells. B-cells internalize their tied antigen and after processing, they present a molecule of the antigen along with a MHC protein to T-cells. The second signal derives from the T-cell in terms of the CD40L. The response of T-cells to signal one is either anergy or deletion towards the (auto-) antigen, while signal two converts a tolerogenic autoreactive lymphocyte into an activated one. Multiple defects in Lupus B- and T-cells have been reported and include hyperreactivity, defects in inducing apoptosis, lowered activation threshold or upregulation of co-stimulatory molecules.

T-cells play numerous roles in the pathogenesis of Lupus Nephritis. They regulate B-cell function and hence antibody formation, their proinflammatory cytokines upregulate adhesion and MHC molecules, recruit macrophages and promote fibrosis. Finally, T-cells directly cause tissue damage via their cytotoxic effectors. They also regulate an isotype switch towards the IgG isotype. Furthermore, imbalances in number has been reported by the means that CD4<sup>+</sup> cells are increased relative to CD8<sup>+</sup> cells and additionally, double negative CD4<sup>-</sup> 8<sup>-</sup> cells are increased in the serum but they can be found in renal lesions, too.

B-cells are appreciated the most for antibody formation but their part in regulation of the immune system and their ability to present antigens is often forgotten. Besides their function as immunoglobulin factories, B-cells activate autoreactive T-cells via antigen presentation and regulate development and activation of dendritic cells. Antibodies are to be held responsible for complement activation and thrombus formation in the glomerular capillaries. Via Fc-receptors on leukocytes or renal parenchymal cells, antibodies cause leukocyte adhesion and the release of various proinflammatory cytokines. It must be noted, that antibodies binding to DNA are important but still, antibodies against many other renal structures can be found and anti-DNA-antibodies are neither necessary nor sufficient to provoke Lupus Nephritis.

Murine models of Lupus Nephritis elucidated a potentially critical role of renal parenchymal cells and proximal tubule epithelial cells. They also function as antigen presenting cells and maintain autoreactivity by regulating CD4<sup>+</sup> T-cells. However, the role of this finding has yet to be characterized in humans.

#### 1.2.2.3. Other contributing factors

Derangements in apoptosis and cell removal by the mononuclear phagocytic cell system also bear important implications regarding the pathogenesis of LN. While a defect in the Fas-ligand of lupus mouse strains brought clear results, its importance in human Lupus Nephritis could not be confirmed. But a very important implication could be detected in the formation of nucleosomes. It was proven that anti-dsDNA antibody formation was impossible as long as the DNA was not linked to histone proteins. As a consequence, antibodies are aimed at histone proteins and they reach their variability by epitope spreading but they are not targeted against DNA in the first place. Along with this finding, the theory that anti-dsDNA antibodies are cross-reactive with proteins of the glomerular basement membrane was reviewed and data showed that complex nucleosome binding to anti-dsDNA antibodies is pivotal for establishing glomerular damage<sup>13</sup>.

Lately, there has been a special interest on the role of adhesion molecules in Lupus Nephritis. Predominantly secreted by endothelial cells on cytokine stimulus, they are pivotal for the invasion of leucocytes into the area of inflammation. The molecules which are investigated best are various selectins (P-, L- or E-selectin) and the vascular cell adhesion molecule 1 (VCAM-1). Besides their role in triggering inflammation and coagulation, data exists claiming their importance in the detection of onset of renal damage due to their very early increase in the course of nephritis in serum and/or urine<sup>14,15</sup>.

### **1.2.3 WHO- Classification**

As mentioned above, renal- and neuropsychiatric involvement are the major predictors determining the outcome in SLE patients. Lupus nephritis can be seen in as much as 60 percent of patients suffering from Lupus. Major risk factors are described above.

Lupus nephritis is divided into six classes and various subclasses as defined by WHO classification. Every clinical and laboratory sign of glomerular damage can be seen but it is not possible to predict the histological stage of disease from clinical presentation alone. The WHO classification was revised in 2003 by the International Society of Nephrology and the Renal Pathology Society Working Group On The Classification Of Lupus Nephritis<sup>16</sup>. The major difference as compared to the original WHO classification is the introduction of shortcuts in order to provide more information to the clinical physician. The letters A and C are used to stage the lesions as “active” (A) or chronic (C) in class III and class IV lupus nephritis, while the letters G and S inform whether a lesion is “global” (G) or sclerotic (S) in class IV nephritis.

Due to the fact that WHO classification is still widely used in scientific papers, it is to be described in here.

#### 1.2.3.1. WHO CLASS I

- a) Normal glomeruli by all techniques
- b) Immunedeposits visible in electron microscopy and/or immunohistochemistry only

This type of “nephritis” shows normal sediment and urinalysis and usually, no signs of renal impairment in serum analysis. The frequency of occurrence lies under one percent of all lupus patients in whom biopsy is performed. Due to its benignity it does not need special attention in treatment.

#### 1.2.3.2. WHO CLASS II - Mesangioproliferative Lupus Nephritis

- a) Mesangial widening and/or mild hypercellularity
- b) Mesangial cell proliferation

Patients typically present with renal hematuria and/or microproteinuria. Cell casts or nephrotic syndrome are seen rarely in this condition which practically never shows renal insufficiency. While it used to get no special attention in treatment in earlier years, a trend can be seen towards early treatment of this condition lately in order to prevent progression. Maybe one of the reasons why this change of attitude has happened is the fact Mycophenolate-Mofetil (MMF) is by far less toxic than its alternative Cyclophosphamide, but still, both drugs show an equal potency. Its frequency of occurrence lies at twenty-six percent in all SLE patients who undergo biopsy.

#### 1.2.3.3. WHO CLASS III - Focal Sclerosing Lupus Nephritis

- a) Active necrotizing lesions
- b) Active and sclerosing lesions
- c) Sclerosing lesions

This class is seen in eighteen percent of renal biopsies and usually goes along with mild or intermediate alterations of the mesangium in terms of hypercellularity and/or capillary widening. Clinically, these patients usually present with urinary casts and proteinuria which leads to a nephrotic syndrome in twenty to thirty percent. Renal deterioration can be seen in up to a quarter of patients and sometimes it is hard to distinguish from WHO class IV nephritis. Despite from that fact, class III nephritis can convert to type IV so that patients exhibiting a type III LN need to be watched carefully and treatment with Cyclophosphamide plus steroids or Mycophenolat-Mofetil (MMF) and steroids should be introduced.

#### 1.2.3.4. WHO CLASS IV - Diffuse Proliferative Lupus Nephritis

- a) With segmental lesions
- b) Active necrotizing lesions
- c) Active and sclerosing lesions
- d) Sclerosing lesions

This entity represents the biopsy's worst case scenario and it is the result of thirty-eight percent of biopsies performed in SLE patients. Those suffering from class IV nephritis can exhibit all kinds of renal symptoms, ranging from renal hypertension to nephritic sediment, deterioration of the renal function, microhematuria and a proteinuria of variable extent, sometimes leading to a nephrotic syndrome. Long-term prognosis is bad if no treatment is initiated and untreated patients are likely to survive two years at most<sup>17</sup>. However, with early treatment and an accurate disease management, the five year survival rate is only little lower as compared to Lupus Nephritis as a whole. Treatment should be initiated as early as possible and it should consist of Cyclophosphamide and steroids or Mycophenolat-Mofetil and steroids. Recently, a small study reached clinical remission in patients not responding to Cyclophosphamide by adding the monoclonal anti-CD20 antibody Rituximab to the therapy scheme<sup>18</sup>.

#### 1.2.3.5. WHO CLASS V - Membranous Lupus Nephropathy

- a) Pure membranous glomerulonephritis
- b) Associated with lesions of category II (a or b)

In literature, class V lesions are either called “glomerulonephritis” or “glomerulopathy” which gives credit to the fact that type Va lesions do not show inflammation. As mentioned above, the pathogenic background is a function loss of the podocytes and the glomerular basement membrane as a consequence of thickening of the basement membrane due to immune complex formation. Patients therefore usually establish a nephrotic syndrome whereas urinalysis and GFR are normal in the early stages of disease. It is present in sixteen percent of kidney biopsies and sometimes class V nephritis is diagnosed prior to the onset of Systemic Lupus Erythematosus. If subendothelial and tubular immune deposits can be found next to the pathognomonic subepithelial lesions, suspicion rises that the nephropathy is associated to SLE. Class V nephritis is characterized by a big variation of the clinical course which leads to the problem of how to treat it. Patients who exhibit symptoms associated with complications, e.g. high initial serum creatinine, massive proteinuria or hypertension should not be withheld toxic medication. On the other hand, with the introduction of potent drugs with low toxicity like MMF and its successors, treatment of all patients with membranous nephropathy is justified.

Due to the massive protein loss which typically goes along with membranous nephropathy, the risk for thrombembolic complication rises because coagulation inhibiting proteins like

antithrombin III, protein C or protein S can not be held back by the glomerular basement membrane and are lost via urine.

#### 1.2.3.6. WHO CLASS VI - Advanced Sclerosing Glomerulonephritis

This entity represents the end-stage of Lupus Nephritis and early treatment of all the other stages is performed in order to prevent the patient from progressing to this stage. The major risk factor, besides non-treatment of Lupus Nephritis in general, is long time renal involvement. Clinically, patients exhibit mild proteinuria, renal hypertension and kidney insufficiency. Treatment usually does not ameliorate the glomerular filtration rate and consists of hemodialysis and kidney transplantation.

#### 1.2.4. Treatment and Outcome of Lupus Nephritis<sup>5:18;19</sup>

Determinants associated with a worse outcome are onset after fifty-five years, childhood onset, hypertension, deterioration of renal function at diagnosis and class IV nephritis. Over the course of time, prognosis has also changed distinctively: the introduction of steroids and immunosuppressive drugs has led to an amelioration of the five-year actual survival rate from 17 percent of class in class IV nephritis from 1953-1959 to 82 percent in the era from 1990-1995.

The alkylating agent Cyclophosphamide was used as standard treatment in LN but at present, it seems to lose this state in favor of Mycophenolat-Mofetil. Cyclophosphamide is categorized as a “radiomimetic” drug for its alterations of the DNA are similar to effect of radiation therapy by forming interstrand and intrastrand DNA cross-links which lead to cell death. Two different regimes, namely high dose intra venous pulse therapy and low dose daily oral application, are used and differ in their toxicity. Low dose daily oral treatment shows significantly more side effects, especially in the urinary tract, increasing the risk of carcinoma of the bladder and bleeding of urinary tract. Regarding the usually very high rate of infections, intra venous pulse therapy is not superior as compared to oral medication. Other important side effects of Cyclophosphamide are increased risk of malignancy and gonadal failure. While fewer cancers appeared in patients who were treated with i.v. pulse therapy, damage of the reproductive organs mainly depends on the cumulative dose received and on the age of the patient but not on the regime of application.

Mycophenolat-Mofetil inhibits the de novo synthesis of purines. Lymphocytes depend heavily on this synthesis so that MMF is a very potent inhibitor of lymphocyte reproduction while other cells are affected less. It is one of the most important drugs in transplant

medicine and it is widely used in this sector. The benefit, as compared to Cyclophosphamide, clearly lies in its significantly less toxicity. Most important side effects are nausea, vomiting and diarrhea, but also anemia and leucopenia are reported. It should not be administered during pregnancy and it is phlebotoxic if given intravenously.

Several studies have been performed comparing these two agents with additional steroid application. But until present, those studies reflect a selected cohort because they mostly included only a small number of patients, and moreover, most of these trials were conducted in Asia. Until now, limited data is available and the studies are best evaluated in reviews and meta-analysis. But despite their limitations, it can be seen that MMF shows an equal or even better performance especially in the stage of remission induction and a significantly better toxic profile as compared to Cyclophosphamide. The long term use of MMF has still to be evaluated. Moreover, the question arises for how long Mycophenolat-Mofetil will be of relevance in the treatment of Lupus Nephritis since many targeted therapies are in various stages of admission and case reports of administration of the monoclonal anti-CD20 antibody Rituximab in severe and therapy resistant patients show promising results<sup>19</sup>.

### **1.3. The problem of assessing the GFR<sup>20,21</sup>**

Plasma concentration of any substance is influenced by multiple factors like enteral uptake or secretion, secretion via sweat, metabolic state and excretion via urine. In the kidneys, glomerular filtration and tubular secretion or reuptake determine the quantity of excretion of a certain substance in the urine.

Glomerular filtration is the initial step of urine formation. The quantity of a certain substance that is filtered by the sum of glomerula in a defined period of time is called glomerular filtration rate (GFR). It is proportional to the permeability of the glomerular wall and the effective filtration pressure. Permeability is influenced by the hydraulic conductivity and the surface area available for filtration. The effective filtration pressure is defined as difference between hydraulic and oncotic pressure gradient.

(Eq. 1)  $GFR = LpS \times (\Delta \text{hydraulic pressure} - \Delta \text{oncotic pressure})$

With  $Lp$  representing unit permeability and  $S$  the surface area of the capillary wall.

To determine the function of the kidney, a substance showing a stable plasma concentration, which is filtered by the glomerula freely and which passes the tubular system un-

changed. In this case, the clearance of this substance is equal to the GFR. But until now, no such marker has been found. In order to evaluate the glomerular filtration rate many different approaches have been established but they are either not performable in clinical practice or their results are influenced by factors other than the GFR.

### **1.2.1. Inulin Clearance**

Inulin is a low molecular polysaccharide which meets all criteria for an ideal substance regarding assessment of renal function, i.e. GFR is equal to the inulin clearance. But this procedure is not practicable due to the considerably high amount of time needed and due to the high costs it would involve.

### **1.2.2. Serum Creatinine**

Serum creatinine derives from muscular protein metabolism and is released to the serum at a more or less constant rate. It is freely filtered by the glomerulum but it gets secreted by the proximal tubule to a certain extent. It correlates inversely with the glomerular filtration rate but in terms of deterioration of the renal function leads to accumulation of creatinine in the serum.

Serum creatinine level is markedly associated with meat intake and protein metabolism. On the one hand, high meat or protein intake causes elevation of the serum creatinine level, whereas on the other hand, meat-free diet can lower the level to as much as 15 per cent. This is to say that diet alone can lead to overestimation as well as underestimation of renal function, which is why serum creatinine should ideally be measured when the patient is fasting.

Despite of respecting protein intake and metabolism, several other facts have to be taken into account when estimating renal function with creatinine accumulation.

Firstly, the estimation is valid only in the steady state when serum creatinine is stable. Fast loss of kidney function, i.e. in acute renal failure, is not accompanied by adequate fast rise of creatinine level because the given time is too short for creatinine to accumulate.

Secondly, the shape of the GFR - serum creatinine curve is important. The relatively narrow range of physiologic or near-physiologic creatinine level covers a relatively wide range of GFR. An apparently slight creatinine increase from 1.0 to 1.5 mg/dl, for example, can represent a deterioration of GFR of 40 ml/min from 120 ml/min to 80 ml/min (“creatinine blind range”). On the other side, an apparently big increase from 6 mg/dl to 12 mg/dl can reflect a minor reduction in GFR from 20 ml/min to 10 ml/min. This suggests on the

one hand that the important major loss of function in beginning renal disease goes along with an initial minor increase of serum creatinine. On the other hand, however, minimal reductions of GFR in progressing renal disease can be detected more easily by following the course of serum creatinine than by following the course of the glomerular filtration rate itself.

### 1.2.3. Creatinine Clearance

The next possible method to assess renal function is to calculate a creatinine clearance from the creatinine levels in serum and urine. Unfortunately, about 10 – 20 per cent of urine creatinine comes from tubule secretion. Interestingly, this problem is cancelled out because the measurement of serum creatinine fails to detect the correct value at about the same extent due to noncreatinine chromogens, which interfere with creatinine in colorimetric reaction. Currently, calculating the creatinine clearance is currently probably the best way to investigate renal function. Its measurement works as follows:

The patient has to collect his urine for 24 hours. From this collection urine volume and creatinine level are assessed. Then, serum creatinine is measured and the clearance is calculated by the formula

$$\text{Eq 2) } 24hCr_{Cl} = (U \times UV) / (S \times T)$$

U= Urine creatinine, UV= Urine volume of 24h collection

S= Serum creatinine, T= Time in minutes (24h x 60min = 1440)

But like all the other methods 24hCr<sub>Cl</sub> has limitations and difficulties, too. Probably the biggest problem is probably accurate collection of the urine. But by calculating the approximate creatinine excretion, physicians have a mathematical tool to roughly proof if a patient has collected properly. Men younger than 50 years excrete about 20-25 mg creatinine per kilogram body weight per day; women of the same age excrete approximately 15-20 mg creatinine per kilogram body weight per day. In older patients creatinine excretion declines due to the decrease in muscle mass and the following formulas encounter this fact.

$$\text{Eq 3) } \textit{Creatinine excretion} = 28 - (\textit{age in years}/6) \textit{ (in men)}$$

$$\text{Eq 4) } \textit{Creatinine excretion} = 22 - (\textit{age in years}/9) \textit{ (in women)}$$

The fact that tubular creatinine secretion rises with the onset of renal disease constitutes a further problem. Thus, urine creatinine level may not reflect glomerular filtration and may be inadequately high. Tubular secretion can increase about 50 percent from its baseline value and can contribute up to 35 percent to the urine creatinine concentration, leading to an overestimation of the glomerular filtration and suggesting good organ function despite severe loss of functional parenchyma. This is a crucial aspect of  $24\text{hCr}_{\text{Cl}}$  because it suggests regarding the creatinine clearance as the upper limit of what the true GFR might be. As a consequence of these circumstances creatinine clearance (and serum creatinine level) should never be the only parameters to assess renal function. These tests should always be seen in context with urinalysis and physical examination.

#### **1.2.4.MDRD-Formula<sup>22</sup>**

In 1999 the Modification of Diet in Renal Disease Study group created different formulas aiming to improve renal diagnostics. Commonly used markers were mathematically brought together into a single formula. It included serum creatinine, serum urea nitrogen, serum albumin and demographic characteristics (age, sex and race) representing metabolic and constitutional differences between subjects of either group.

$$(Eq\ 5)\ GFR = 170 \times (\text{serum creatinine concentration})^{-0,999} \times (\text{age})^{-0,176} \times (0,762\ \text{if patient is female}) \times (1,180\ \text{if patient is black}) \times (\text{serum urea nitrogen})^{-0,170} \times (\text{serum albumin concentration})^{+0,318}$$

Serum creatinine concentration in mg/dl, serum urea nitrogen in mg/dl and serum albumin concentration in g/dl

Benefits of this formula are the cheap and easy assessment as well as good estimation of the kidney function in “standard patients” as represented by middle aged Caucasians without drastic alterations of serum albumin level, i.e. due to liver disease or nephrotic syndrome, or patients who do not cover a steady state of creatinine balance as for example in acute renal failure as has been noted by the authors of this study. It is also inaccurate for people younger than 18 years or older than 70 years. The validity of the MDRD formula for coloured persons is also restricted because only 12% of the study cohort were blacks.

### **1.2.5. Cystatin C<sup>1,2</sup>**

This potent cysteine proteinase inhibitor is a low molecular protein with a weight of 13kD. It is produced at a constant rate by all nucleated cells and filtered in the glomerulum freely. Cystatin C gets reabsorbed in the tubule system where it is metabolized and eliminated. Many studies have been performed investigating probable influences upon the serum level of this marker. In a large cohort (n=212) Cystatin C was independent from age, gender, glucose tolerance, proteinuria, complications of systemic inflammation, SLE and steroids suggesting a good practicability. This study included twenty patients with Lupus Nephritis and until present, it is the only trial investigating Cystatin C in Lupus. However, no study has yet been focusing on LN alone.

Due to the fact that this molecule was independent upon multiple factors in various trials it is usually regarded as a marker which reliably reflects the GFR. But recently, reports have been published severely endangering the thesis. One trial suggests that the serum level is linked to body composition by finding a clear correlation towards the lean mass and other reports claim a dependence of Cystatin C upon thyroid function<sup>23;2425</sup>

Due to the fact that SLE is a process which can virtually affect the whole body, possible influences of many different physiological systems and drugs on the Cystatin C level can be investigated in this specific condition.

### **1.2.6. Proteomics: Future and Solution of the Problem?<sup>26</sup>**

The aim of the introduction so far was to make clear that on the one hand, immune response in SLE and Lupus Nephritis is highly complex but still selective which implicates the heterogeneity of Lupus, and on the other hand, that exact measurement of the renal function is a myth more than a matter of fact. Possible help for both understanding and evaluating renal function and Systemic Lupus Erythematosus is provided novel methods like proteomics or functional genomics. Proteomics uncovers both the structure and function of a various proteins simultaneously using numerous different methods like gel electrophoresis, immunoblotting or enzymatic and metabolic assays. With the help of this method, proteomic profiles could be established assessing the location and extent of renal injury, discerning nephritis subtypes, giving information about prognosis or monitoring the response of the disease to treatment. Many studies have been performed or are underway using this method in order to establish highly specific and sensitive panels for all kinds of kidney injures, even on Lupus Nephritis<sup>27;28</sup>

However, taking into account that this method is still in the beginning of its career, it is way too early to specify its value. Its usage is accompanied by numerous inconveniences such as costs or the fact that it is not yet feasible in the daily clinical practice. Moreover, proteomics has a massive output and interpreting the results is a difficult task.

#### **1.4. Background and aim of the study**

Lupus nephritis (LN) is one of the major problems in patients suffering from Systemic Lupus Erythematosus but still no marker has been found which indicates renal impairment at an early stage<sup>29,30</sup>. Until present, proteinuria and the presence of urinary casts are regarded as the first sign of LN but since early diagnosis and treatment are crucial for the outcome of LN<sup>31</sup> it is highly desirable to find a marker which detects renal impairment in LN as early as possible. Cystatin C and is a very interesting candidate to reveal early renal impairment<sup>1,2,32</sup> and assessing its relevance is aim of this study.

The following questions are of interest.

- Is Cystatin C superior in estimating the GFR as compared to MDRD or serum Creatinine?
- Is Cystatin C really independent upon inflammation and body composition?
- Do any Lupus-specific complications influence the serum level of Cystatin C
- Which marker reflecting renal function is influenced most or least by inflammation and body composition?
- Which marker shows the best performance in the Creatinine blind range?
- Which marker shows the best performance regarding moderate restriction of the kidney function?

## **2. Materials and Methods**

### **2.1. Patient recruitment and Ethics Commission approval**

Patients were collected at the Departments of Rheumatology and Nephrology of the Medical University of Graz. Each patient suffered from a Systemic Lupus Erythematosus as demanded by ACR criteria. The collection of the samples required informed consent. The application form for this clinical trial was filed to the Ethics Commission of the Medical University of Graz and granted on October 22<sup>nd</sup> 2007 (EK-number 19-045 ex 07/08).

In every patient, multiple blood tests were performed and morning portions of urine were collected to conduct urinalysis. Some patients underwent a twenty-four hour urine collection from which protein excretion and Creatinine Clearance were determined. Moreover, about 8ml of serum and urine were collected from each patient; the serum was centrifuged at 5000 rpm for five minutes and finally, both urine and serum was frozen at -20°C.

## **2.2. Statistical Analysis**

The data was statistically analyzed using SPSS (Statistical Package for Social Sciences) version 9.0 under Windows Vista. For the correlation analysis the subjects were divided into two groups (SLE without Lupus Nephritis and SLE with Lupus Nephritis). Every patient in who not all relevant data was obtained was excluded from analysis.

### **2.2.1 Cohort formation and comparison**

For most analysis, the study cohort was divided into two subgroups where Group 0 was defined as SLE without LN and Group 1 was defined as SLE with Lupus Nephritis. To prove their comparability, descriptive analysis was performed and boxplots of the most important markers were drawn. All parameters were tested regarding their distribution. Histograms were drawn and the results were compared with the kurtosis of the parameters. In normally distributed variables, two sided t-test was performed while in parameters which did not show normal distribution, Mann-Whitney-U-Test was conducted.

By the means of keeping a better overview, analysis was divided into several parts reflecting the different groups of parameters, i.e. parameters describing renal function, body composition and inflammation. Variables other than that were summarized at the very beginning.

### **2.2.2. Renal markers and GFR**

Cystatin C, MDRD, serum Urea and serum Creatinine were tested regarding their correlation with the estimated GFR where 24h Creatinine Clearance was used as referential marker. In order to determine a statistically significant difference in any of these markers, a two sided t-test or a Mann-Whitney-U-Test was performed depending on the distribution pattern of the markers.

Another task was to assess the reliability of the renal markers regarding moderate restriction of the GFR and the Creatinine blind range. Moderate restriction of the GFR was defined as 24h Creatinine Clearance between 60ml/min and 95ml/min. Creatinine blind range was defined as serum Creatinine greater than 0,8mg/dl and lower than 1,3 mg/dl. Correlation analysis was conducted to reveal the performance of the markers regarding these tasks.

### **2.2.3. Determining the dependence of renal biomarkers upon inflammation**

In order to prove whether the Cystatin C value is influenced by inflammation, correlation analysis was performed. To assess if certain markers influence the estimation of the GFR, the cohort was divided into two cohorts. In group 0 all subjects with pathological serum level of the inflammation marker were included, while those with physiological serum value were introduced to group 1. Correlation analysis was performed to show the impact of the pathological serum value on estimating the GFR.

### **2.2.4. Determining the dependence of renal biomarkers upon metabolism**

As mentioned above, one major problem of estimating the GFR by either MDRD formula or serum Creatinine level is the influence of the metabolic state upon these markers. In order to assess the superiority of Cystatin C as compared to MDRD and serum Creatinine, correlation analysis was performed. The impact of pathological Transthyretin levels upon the renal markers was assessed by dividing the cohorts into two groups with either physiological or pathological serum Transthyretin level. Correlation analysis was conducted to show the influence of decreased serum Transthyretin level upon renal markers.

### **2.2.5. Determining the dependence of renal markers upon thyroid function**

In order to prove whether thyroid function influences one of the investigated markers, correlation analysis was performed. Key statistics were assessed with descriptive analysis and Scatter plots were drawn to graphically emphasize the relation between fT3 and Cystatin C. Unfortunately, thyroid hormones were attained after the collection of the patient had been finished. This explains why they could be gained in only seventeen patients.

## **3. Results**

### **3.1. Cohort formation and comparison**

### **3.1.1. Age, Gender, Drugs and Complicating Diseases**

All together, twenty-four patients were included in the study from which sixteen suffered from SLE with Lupus Nephritis. Median age was thirty-five years in the SLE only group as opposed to thirty-six years in the nephritis group.

From those patients suffering from LN, five showed type two, or mesangioproliferative nephritis, three patients showed type three, or focal-sclerosing nephritis, six patients showed type four, or diffuse proliferative nephritis, no patient showed type five or diffuse membranous nephritis and in two patients, histological WHO-classification could not be obtained. Lupus nephritis was biopsy proven the earliest in 1991 and the latest in August 2006, the median duration of Lupus Nephritis being seven years.

Sixteen patients were treated with Cellcept (MMF) for more than at least 3 months (dose ranged from 500mg per day to 3000mg per day), eleven patients received steroids (2,5mg per day to 25mg per day). Chloroquin was administered in five subjects where in three cases it was additional to MMF, in one patient it was additional to Azathioprin and Cyclosporin A and in one subject it was the only disease modifying anti-rheumatic drug. One patient received Methotrexat additionally to MMF and Prednisolon.

Complicating diseases were antiphospholipid syndrome in eight patients and hypertension in nine subjects. Five patients received thyroid hormones due to latent or manifest hypothyreosis and one patient suffered from M. Basedow and was treated with Prothiucil.

All together, three male patients were included in the study, all of them suffering from Lupus Nephritis. All patients were of Caucasian origin.

### **3.1.2. Renal parameters and 24h Protein Excretion**

The distribution and the number of samples gained within each parameter can be seen in **Table 1** and in the boxplots **Figure 1** and **Figure 2**.

Statistiken

Group			CreaCl	MDRD	CysC	Serum Crea	UREA	U-Eiw/24h
SLE without Nephritis	N	Gültig	8	8	8	8	8	8
		Fehlend	0	0	0	0	0	0
		Mittelwert	107,1250	92,0475	.9488	.8050	26,75	211,6533
		Median	104,8500	89,4050	.8950	.8000	25,00	135,9750
		Varianz	225,2907	73,4024	.0312	.0213	132,79	53757,16
		Schiefe	,557	,507	1,648	,176	1,352	2,722
		Standardfehler der Schiefe	,752	,752	,752	,752	,752	,752
		Kurtosis	-,899	-1,599	2,963	-1,010	2,667	7,549
		Standardfehler der Kurtosis	1,481	1,481	1,481	1,481	1,481	1,481
		Minimum	90,10	83,16	.79	.61	13	76,50
		Maximum	131,90	105,24	1,33	1,01	51	780,00
SLE with Nephritis	N	Gültig	16	16	16	16	16	16
		Fehlend	0	0	0	0	0	0
		Mittelwert	88,7063	78,3888	.9269	1,0131	32,38	432,6106
		Median	87,2000	75,1100	.8200	.8600	26,00	169,5000
		Varianz	1033,9340	705,9495	.1127	.3257	482,12	510639,4
		Schiefe	,312	-,470	1,947	3,261	3,064	2,799
		Standardfehler der Schiefe	,564	,564	,564	,564	,564	,564
		Kurtosis	1,154	,630	4,311	11,622	10,444	8,110
		Standardfehler der Kurtosis	1,091	1,091	1,091	1,091	1,091	1,091
		Minimum	24,00	18,55	.58	.59	16	42,42
		Maximum	161,80	125,35	1,91	3,02	108	2769,20

**Table 1) Descriptive statistics of renal markers**

The mean and median values for Creatinine Clearance were pathological in the nephritis group (mean= 88.7; median= 87.2) but in the SLE-only group, however, Creatinine Clearance was in the physiologic range, represented by a mean = 107.1 and median = 104.9. This difference is also reflected by the MDRD formula. The mean and median MDRD values in the nephritis group were in the pathologic range defined as <80ml/min with median being 75.1ml/min and mean being 78.4ml/min, respectively. Figures in the SLE-only group were slightly higher with a mean of 92.1ml/min and median 89.4ml/min.

Ten patients in the nephritis group had a decreased Creatinine Clearance from who eight showed a reduction of MDRD but only four had an increase of the serum Cystatin C level. In contrast, three subjects of the SLE-only cohort presented with a 24hCr<sub>Cl</sub> ranging from 90ml/min to 95ml/min. One of these patients showed a 24hCr<sub>Cl</sub> of 90.1ml/min, MDRD was at 85.3ml/min and Cystatin C 0.81mg/l. Urinalysis revealed microhematuria but no casts while no noteworthy albumin concentration was detected in 24h urine collection.

Patient two had a Creatinine Clearance of 92.7ml/min, MDRD was at 91.94ml/min and Cystatin C was 0.99mg/l, exceeding the threshold value. Urinalysis showed no pathological sediment but urine collection revealed a microproteinuria of 145mg/die. Furthermore, this patient suffered from a common cold disease and presented a CRP of 85.4mg/l.

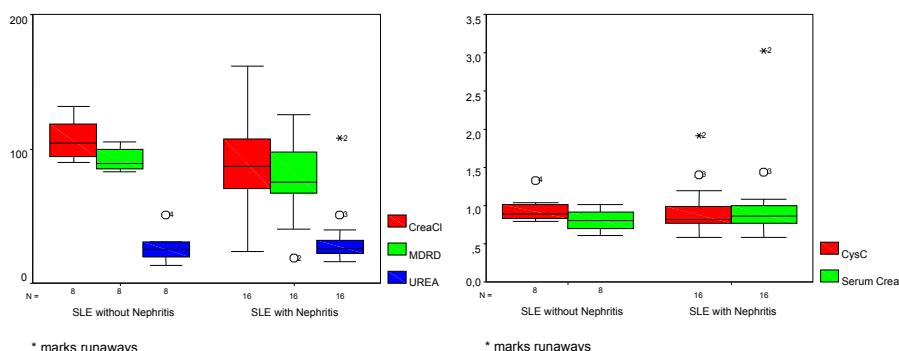
The last of these patients presented with a 24hCr<sub>Cl</sub> of 95.0ml/min, MDRD of 101.1ml/min but a Cystatin C value of 1.33mg/l.

None of them showed a pathologic MDRD value (85.34ml/min, 91.94ml/min and 101.1ml/min respectively) but two of them had a Cystatin C greater than 95mg/l (0.99mg/l and 1.33mg/l, respectively)

Mean Cystatin C level was lower in the nephritis cohort, reflected by a median of 0.82mg/l and a mean of 0.93mg/l as compared to a mean of 0.94mg/l and a median of 0.89mg/l in the SLE-only cohort. Three patients in group 0 had a pathologic Cystatin C value despite low normal Creatinine Clearance and MDRD. In contrast to that, only four subjects in the nephritis cohort showed pathologic Cystatin C values with only three of them having an impaired renal function with MDRD <80ml/min and 24hCr<sub>Cl</sub> of <95ml/min.

Regarding twenty-four hour protein excretion, a somewhat big difference of the mean value could be detected while the medians did not follow that trend. The variance was big in both groups reflecting the heterogeneity of protein excretion in both cohorts. Importantly, both mean and median in the SLE-only group were in the pathologic range indicating microproteinuria in some of these patients.

In serum creatinine and serum urea, all median and mean values are in the physiologic range. The boxplots in **Figure 1** and **Figure 2** additionally emphasize the big variance in all parameters of the nephritis cohort.



**Figure 1) Boxplots of Creatinine Clearance, MDRD and serum Urea in both cohorts**  
**Figure 2) Boxplots of Cystatin C and serum Creatinine in both cohorts**

Apart from the comparison of means and medians, the investigation of the differences among the variances between the two cohorts revealed important information, too. Throughout all parameters analyzed, a remarkably greater variance in the LN group could be seen, which derives from the fact that patients in clinical remission from nephritis do not necessarily show impaired renal function. Since LN patients of all stages of disease and WHO classification have been included, the renal function among the subjects in group 1 varies distinctively. An impact can be seen in the low significance levels of the figures as described below.

As suspected, the nephritis group showed worse renal performance. However, no parameter reached statistical significance defined as  $p < 0.05$  and  $p < 0.01$  defining high statistical significance, respectively. As mentioned above, kurtosis and histograms were the determinants whether T-Test or Mann-Whitney-U-Test was performed. Only  $24hCr_{cl}$  and MDRD were regarded as normally distributed, all other parameters showed a non-parametric distribution.

**Gruppenstatistiken**

Group	N	Mittelwert	Standardabweichung	Standardfehler des Mittelwertes
CreaCl	SLE without Nephritis	8	107,1250	15,0097
	SLE with Nephritis	16	88,7063	32,1548
MDRD	SLE without Nephritis	8	92,0475	8,5675
	SLE with Nephritis	16	78,3888	26,5697

**Test bei unabhängigen Stichproben**

		Levene-Test der Varianzgleichheit		T-Test für die Mittelwertgleichheit						
		F	Signifikanz	T	df	Sig. (2-seitig)	Mittlere Differenz	Standardfehler der Differenz	95% Konfidenzintervall der Differenz	
									Untere	Oberer
CreaCl	Varianzen sind gleich	1,921	,180	1,526	22	,141	18,4188	12,0673	-6,6073	43,4148
	Varianzen sind nicht gleich			1,912	21,978	,069	18,4188	9,6324	-1,5587	38,3162
MDRD	Varianzen sind gleich	4,328	,049	1,404	22	,174	13,6588	9,7277	-6,5153	33,8328
	Varianzen sind nicht gleich			1,871	20,031	,076	13,6588	7,3005	-1,5683	28,8858

**Table 2) Group statistics for Creatinine Clearance and MDRD regarding T-Test**

**Table 3) T-Test for Creatinine Clearance and MDRD**

**Tables 2 and 3** show the t-test for  $24hCr_{cl}$  and MDRD. Creatinine Clearance was closer than MDRD to reach statistical significance with  $p=0,141$  as compared to  $p=0,174$  for MDRD, respectively. Levene test indicates that MDRD is not normally distributed with a significance level of  $p=0.049$ . However, the specificity of this test is low and furthermore, t-test for different variances does not reach statistical significance, too. This can be seen in the p-value in the section “Varianzen sind nicht gleich”.

**Ränge**

Group	N	Mittlerer Rang	Rangsumme
CysC	SLE without Nephritis	8	14,69
	SLE with Nephritis	16	11,41
	Gesamt	24	117,50
Serum Crea	SLE without Nephritis	8	10,44
	SLE with Nephritis	16	13,53
	Gesamt	24	83,50
UREA	SLE without Nephritis	8	11,38
	SLE with Nephritis	16	13,06
	Gesamt	24	209,00
U-Eiw/24h	SLE without Nephritis	8	11,88
	SLE with Nephritis	16	12,81
	Gesamt	24	95,00

Statistik für Test<sup>b</sup>

	CysC	Serum Crea	UREA	U-Eiw/24h
Mann-Whitney-U	46,500	47,500	55,000	59,000
Wilcoxon-W	182,500	83,500	91,000	95,000
Z	-1,073	-1,011	-,553	-,306
Asymptotische Signifikanz (2-seitig)	,283	,312	,581	,759
Exakte Signifikanz [2*(1-seitig Sig.)]	,291 <sup>a</sup>	,320 <sup>a</sup>	,610 <sup>a</sup>	,787 <sup>a</sup>

a. Nicht für Bindungen korrigiert.

b. Gruppenvariable: Group

**Table 4) Rank statistics for Mann-Whitney-U Test regarding Cystatin C, serum Creatinine, serum Urea and Twenty-four hour protein excretion**

**Table 5) Mann-Whitney-U Test for Cystatin C, serum Creatinine, serum Urea and Twenty-four hour protein excretion**

**Tables 4 and 5** show the parameters analyzed with Mann-Whitney-U-Test. Each marker missed the significance level with  $p=0.283$  for Cystatin C marking the best performance. Serum Creatinine with  $p=0.312$ , serum Urea with  $p=0.581$  and twenty-four hour protein excretion with  $p=0.759$  showed markedly worse significance levels. In order to receive better comparability among the different tests, only those data where  $24hCr_{Cl}$  was obtained were included.

### 3.1.2. Markers reflecting Body Composition

Statistiken

Group			BMI	Transthyr	SerumAlb	TotProt	
SLE without Nephritis	N	Gültig	8	8	8	8	
		Fehlend	0	0	0	0	
		Mittelwert	23,0837	.21700	3,875	7,713	
		Median	22,7400	.19950	3,850	7,550	
		Varianz	6,3715	.00527	6,786E-02	,496	
		Schiefe	1,735	1,096	-,606	,283	
		Standardfehler der Schiefe	,752	,752	,752	,752	
		Kurtosis	4,183	1,301	,177	-1,605	
		Standardfehler der Kurtosis	1,481	1,481	1,481	1,481	
		Minimum	20,20	.132	3,4	6,8	
		Maximum	28,70	.361	4,2	8,7	
	SLE with Nephritis	N	Gültig	16	16	16	16
			Fehlend	0	0	0	0
		Mittelwert	22,9569	.26775	4,150	7,625	
		Median	23,5000	.25700	4,250	7,650	
		Varianz	9,0260	.00257	,117	,335	
		Schiefe	,050	,399	-,182	-,893	
		Standardfehler der Schiefe	,564	,564	,564	,564	
		Kurtosis	-1,404	-,702	-,375	1,060	
		Standardfehler der Kurtosis	1,091	1,091	1,091	1,091	
		Minimum	18,70	.184	3,5	6,2	
		Maximum	28,08	.363	4,8	8,4	

**Table 6) Descriptive analysis regarding Body Mass Index, Transthyretin, serum Albumin and serum Total Protein count**

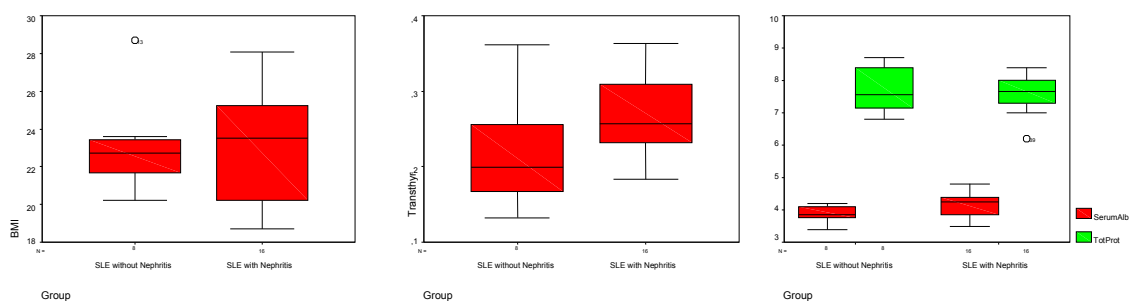
The next task was to describe possible differences regarding body composition between the two cohorts. Mean and median values of all parameters included in the analysis were in the

physiological range except for the median value of Transthyretin which was with 0.199g/l slightly under the threshold value of 0.200g/l. The distribution can be seen at best in the boxplots, **Figures 3-5**. Groups show a good comparability with a trend towards lower levels in the SLE-only cohort in nearly every parameter investigated.

The Body Mass Index showed similar means and medians among the two cohorts with a greater variance of data in the nephritis group. Taking a look at minimum and maximum levels indicates that no patient included in this trial suffered from severe obesity or severe anorexia.

All together, five patients showed a decrease in Transthyretin level (<0.2g/l) whereas four subjects were from the SLE-only group. Four of them showed microproteinuria (>110mg/die) and three subjects had a CRP greater than 10mg/l.

Mean and median value for serum Albumin was in the physiologic range in either group with a trend towards lower levels in the SLE-only group. Yet, only one patient had a decreased Albumin level but with 3,4g/dl the threshold value was exceeded by only 0.1g/dl. This patient also showed a decrease in Transthyretin level as well as a CRP of 15mg/l and a microproteinuria (189mg/24h) but urinalysis revealed no pathologic sediment.



**Figure 3) Boxplots for the Body Mass Index in both groups**

**Figure 4) Boxplots for Transthyretin in both groups**

**Figure 5) Boxplots for serum Albumin and serum Total Protein in both groups**

Interestingly, only one nephritis patient presented a decreased serum total protein level indicating protein loss due to renal damage. In addition, serum analysis revealed increased Cholesterol and Triglyceride levels, near-pathologic serum Albumin (3,5g/dl) and total protein in twenty-four hour urine collection was 2,8g/die suggesting nephrotic syndrome. Surprisingly, the patient showed good renal function with 24hCr<sub>Cl</sub> being 128ml/min, MDRD being 97.7ml/min but however, Cystatin C value was near-pathologic with 95mg/l. In contrast to these findings, two patients in group 0 and two patients with Lupus Nephritis exceeded the critical upper level of total serum protein concentration. Two of them showed elevated CRP and Fibrinogen values suggesting that this finding might be reactive to infec-

tion. None of the patients suffered from a monoclonal gammopathy as proven by serum electrophoresis.

Kurtosis and histograms revealed that each of the parameters was normally distributed. As shown in **Tables 7 and 8** no marker reached a statistically significant p-value, but however, Transthyretin with p=0.58 and serum Albumin with p=0.59 came very close to statistical significance. The Body Mass Index and serum total protein were fairly equal in both groups, which is also reflected by the close median and means.

**Gruppenstatistiken**

Group	N	Mittelwert	Standardabweichung	Standardfehler des Mittelwertes
BMI	SLE without Nephritis	8	23,0837	2,5242
	SLE with Nephritis	16	22,9569	3,0043
Transthyr	SLE without Nephritis	8	.21700	.07261
	SLE with Nephritis	16	.26775	.05071
SerumAlb	SLE without Nephritis	8	3,875	,260
	SLE with Nephritis	16	4,150	,343
TotProt	SLE without Nephritis	8	7,713	,704
	SLE with Nephritis	16	7,625	,579

**Test bei unabhängigen Stichproben**

		Levene-Test der Varianzgleichheit		T-Test für die Mittelwertgleichheit						
		F	Signifikanz	T	df	Sig. (2-seitig)	Mittlere Differenz	Standardfehler der Differenz	95% Konfidenzintervall der Differenz	
									Untere	Oberer
BMI	Varianzen sind gleich	2,665	,117	,102	22	,919	,1269	1,2386	-2,4417	2,6955
	Varianzen sind nicht gleich			,109	16,553	,915	,1269	1,1664	-2,3392	2,5929
Transthyr	Varianzen sind gleich	,947	,341	-2,001	22	,058	-.05075	.02536	-.10335	.00185
	Varianzen sind nicht gleich			-1,773	10,537	,105	-.05075	.02863	-.11411	.01261
SerumAlb	Varianzen sind gleich	1,193	,287	-1,993	22	,059	-.275	,138	-.561	1,123E-02
	Varianzen sind nicht gleich			-2,187	18,043	,042	-.275	,126	-.539	-1,08E-02
TotProt	Varianzen sind gleich	1,142	,297	,325	22	,748	8,750E-02	,269	-.471	,646
	Varianzen sind nicht gleich			,304	11,902	,766	8,750E-02	,288	-.540	,715

**Table 7) Group statistics for Body Mass Index, Transthyretin, serum Albumin and serum total protein  
Table 8) T-test for Body Mass Index, Transthyretin, serum Albumin and serum total protein**

### 3.1.3. Inflammatory parameters and complement

Finally, the groups were compared regarding the inflammatory state of their patients. The most important figures of descriptive analysis can be seen in **Table 9** and in the boxplots of **Figure 6-8**. It is crucial to mention that differential blood count could not be obtained in one subject of the SLE-only group.

Statistiken

Group			CRP	WBC	NEU	LY	C3	C4
SLE without Nephritis	N	Gültig	8	7	7	7	7	7
		Fehlend	0	1	1	1	1	1
		Mittelwert	27,688	5,9357	13,914	,871	,90343	,14057
		Median	13,050	5,9300	4,900	,700	,99000	,14600
		Varianz	1095,293	1,3399	613,338	,276	,08304	,00439
		Schiefe	1,259	,253	2,631	,893	-,004	-,355
		Standardfehler der Schiefe	,752	,794	,794	,794	,794	,794
		Kurtosis	-,116	-,402	6,938	-,738	-,251	-1,325
		Standardfehler der Kurtosis	1,481	1,587	1,587	1,587	1,587	1,587
		Minimum	2,8	4,33	3,1	,3	,490	,051
		Maximum	85,4	7,77	70,0	1,7	1,350	,218
	SLE with Nephritis	N	Gültig	16	16	16	16	16
		Fehlend	0	0	0	0	0	0
		Mittelwert	3,675	5,7394	3,994	1,244	,91756	,17244
		Median	1,600	5,2750	3,650	1,300	,93000	,15500
		Varianz	26,685	1,5670	2,082	7,596E-02	,03038	,00640
		Schiefe	2,026	1,115	1,642	-,458	-,069	,281
		Standardfehler der Schiefe	,564	,564	,564	,564	,564	,564
		Kurtosis	4,535	1,687	4,150	1,102	-1,493	-1,037
		Standardfehler der Kurtosis	1,091	1,091	1,091	1,091	1,091	1,091
		Minimum	,0	3,83	1,9	,6	,670	,058
		Maximum	19,0	8,97	8,2	1,7	1,200	,309

Table 9) Descriptive analysis for C-reactive protein, White Blood Count, absolute Neutrophile count, absolute Lymphocyte count, Complement factor 3 and Complement factor 4

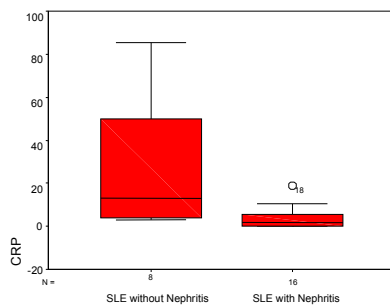


Figure 6

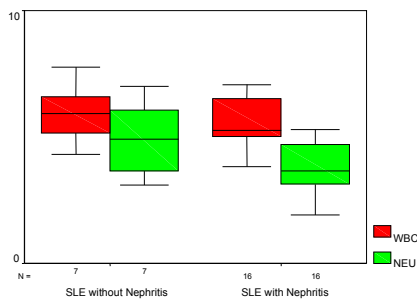


Figure 7

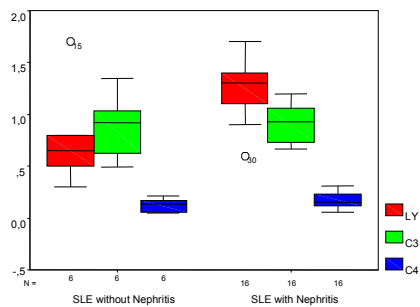


Figure 8

Figure 6) Boxplot for C-reactive protein in both groups

Figure 7) Boxplots for White Blood Count and absolute Neutrophile Count in both groups

Figure 8) Boxplots for absolute Lymphocyte count, Complement factor 3 and Complement factor 4 in both groups

Figures were drawn in three parts because of the different ranges of the parameters. Those with similar scaling were put together and CRP is depicted separately. It is important to mention that runaways are not shown in the Neutrophils graph (Figure 7) in order to preserve a better overview, while they are included in the CRP picture in Figure 6.

Comparing the mean and median CRP values reveals a major difference among the cohorts with a mean of 27.7mg/l, a median of 13.3mg/l in Group 0 and a mean of 3.7mg/l with a mean of 1.6mg/l in the nephritis group, the threshold value of 8mg/l being clearly exceeded by the lupus-only cohort. This can be possibly explained by the fact that two of the eight patients showed high pathologic values of 73.7mg/l and 85.4mg/l, while the highest CRP value was 19.1 in the nephritis group.

Along with CRP, the mean absolute Neutrophile count was higher in the SLE-only cohort, whereas the medians did not really differ.

White Blood Count was comparable between both groups with all mean and median values being in the physiologic range.

Absolute Lymphocyte count revealed decreased values in five subjects of the SLE-only group (mean= 0.87G/l, median 0.7G/l), while median and mean lymphocyte count in the nephritis group was low, but still normal. Only two persons in group 1 showed lymphopenia.

Similar to this result, the complement factors were also in the lower physiologic range. C3 showed a relatively big variance in the non-nephritis group as compared to group 1, while C4 was rather homogenous in both groups investigated.

Ränge					Statistik für Test <sup>b</sup>				
Group		N	Mittlerer Rang	Rangsumme		CRP	WBC	NEU	C4
CRP	SLE without Nephritis	6	16,17	97,00	Mann-Whitney-U	20,000	43,000	35,000	33,000
	SLE with Nephritis	16	9,75	156,00	Wilcoxon-W	156,000	179,000	171,000	54,000
	Gesamt	22			Z	-2,086	-,369	-,960	-1,106
WBC	SLE without Nephritis	6	12,33	74,00	Asymptotische	,037	,712	,337	,269
	SLE with Nephritis	16	11,19	179,00	Signifikanz (2-seitig)				
	Gesamt	22			Exakte Signifikanz				
NEU	SLE without Nephritis	6	13,67	82,00	[2*(1-seitig Sig.)]	,040 <sup>a</sup>	,747 <sup>a</sup>	,367 <sup>a</sup>	,294 <sup>ii</sup>
	SLE with Nephritis	16	10,69	171,00					
	Gesamt	22							
C4	SLE without Nephritis	6	9,00	54,00					
	SLE with Nephritis	16	12,44	199,00					
	Gesamt	22							

a. Nicht für Bindungen korrigiert.

b. Gruppenvariable: Group

**Table 10) Rank statistics of C-reactive protein, White Blood Count, absolute Neutrophile count and Complement factor 4 for Mann-Whitney-U Test**

**Table 11) Mann-Whitney-U Test for C-reactive protein, White Blood Count, absolute Neutrophile count and Complement factor 4**

**Tables 10 and 11** show the results of the Mann-Whitney-U-Test performed for the non-parametric markers as investigated by histograms and kurtosis. Only those patients were included, in whom complete data was attained. In one subject, differential blood count was not obtained and in one subject complement factors were not available. These two were excluded from analysis, so that the total number of patients included in the SLE-only cohort sank from eight to six. CRP, absolute Neutrophile count (NEU), C4 and White Blood Count (WBC) clearly showed non-normal distribution. C-reactive protein reached statistical significance with  $p=0.037$  indicating markedly higher levels in group 0. In contrast to

this finding, WBC, C4 and Neutrophile count were fairly equal in both groups and therefore, they failed to reach significance.

Absolute Lymphocyte count and C3 were regarded as normally distributed and results of the T-Test are depicted in **Table 12 and 13**. As can be seen, the SLE-only cohort presented with statistically significant lower lymphocytes ( $p=0.009$ ), whereas no significant difference in the C3 values among the two groups could be detected ( $p=0.787$ ).

Group	N	Mittelwert	Standardabweichung	Standardfehler des Mittelwertes
LY SLE without Nephritis	6	.767	.489	.199
LY SLE with Nephritis	16	1.244	.276	.069
C3 SLE without Nephritis	6	.88900	.31288	.12773
C3 SLE with Nephritis	16	.91756	.17431	.04358

		Levene-Test der Varianzgleichheit		T-Test für die Mittelwertgleichheit						
		F	Signifikanz	T	df	Sig. (2-seitig)	Mittlere Differenz	Standardfehler der Differenz	95% Konfidenzintervall der Differenz	
									Untere	Oberer
LY	Varianzen sind gleich	1,209	,285	-2,918	20	,009	-.477	.163	-.818	-.136
	Varianzen sind nicht gleich			-2,261	6,235	,063	-.477	.211	-.989	.035
C3	Varianzen sind gleich	3,853	,064	-,274	20	,787	-.02856	.10407	-.24565	.18852
	Varianzen sind nicht gleich			-,212	6,204	,839	-.02856	.13496	-.35619	.29907

**Table 12) Group statistics of absolute Lymphocyte count and Complement factor 3 for T-Test**  
**Table 13) T-Test for absolute Lymphocyte count and Complement factor 3**

## 3.2. Influences on Renal Markers

### 3.2.1. Renal Markers and Proteinuria

Correlation analysis of renal markers was performed with list wise exclusion of missing data. The results can be seen in **Table 14**.

In the SLE-only cohort, only the correlation of Cystatin C and serum Urea reached high statistical significance with  $r=0.835$  and  $p=0.01$ , respectively. No other correlation was statistically significant. The follow-ups were MDRD - serum Creatinine with  $r=-0.683$  and  $p=0.062$  on the one hand, and  $24hCr_{Cl}$  - serum Urea with  $r=-0.649$  and  $p=0.81$  on the other hand. The performances of MDRD and CysC regarding Creatinine Clearance were both far away from statistical significance with Cystatin correlating slightly better ( $r=0.11$  for MDRD versus  $r=-0.35$  for CysC). Moreover, Cystatin C and MDRD showed with a  $r=0.161$  a very poor correlation with each other.

In the nephritis cohort, the correlation among each parameter showed high statistical significance. The best correlation with  $24hCr_{Cl}$  was shown by the MDRD formula ( $r=0.876$ ;  $p<0.000$ ) and Cystatin C ( $r=-0.73$ ;  $p=0.001$ ). But serum Creatinine ( $r=-0.72$ ;  $p=0.002$ ) and

serum Urea (with  $r=-0.65$ ;  $p=0.006$ ) also showed a good correlation rate with the Creatinine Clearance. Importantly, Cystatin C shows the best correlations with every other marker analyzed and outdid the MDRD-formula even in the correlation among serum Urea and serum Creatinine, although both of them are included in its calculation.

P-values and correlation coefficients of the twenty-four hour protein excretion (U-Eiw/24 in Figure 14) were very low in both cohort and every single parameter suggesting that all these markers are independent upon proteinuria.

Korrelationen			CreaCl	MDRD	CysC	Serum Crea	UREA	U-Eiw/24h
SLE without Nephritis	CreaCl	Korrelation nach Pearson	1,000	,112	-,352	,063	-,649	-,031
		Signifikanz (2-seitig)	.	,793	,393	,881	,081	,441
		N	8	8	8	8	8	8
	MDRD	Korrelation nach Pearson	,112	1,000	,161	-,683	,098	-,248
		Signifikanz (2-seitig)	,793	.	,703	,062	,817	,553
		N	8	8	8	8	8	8
	CysC	Korrelation nach Pearson	-,352	,161	1,000	-,129	,835**	-,158
		Signifikanz (2-seitig)	,393	,703	.	,760	,010	,708
		N	8	8	8	8	8	8
	Serum Crea	Korrelation nach Pearson	,063	-,683	-,129	1,000	-,184	,125
		Signifikanz (2-seitig)	,881	,062	,760	.	,662	,769
		N	8	8	8	8	8	8
	UREA	Korrelation nach Pearson	-,649	,098	,835**	-,184	1,000	-,034
		Signifikanz (2-seitig)	,081	,817	,010	,662	.	,937
		N	8	8	8	8	8	8
	U-Eiw/24h	Korrelation nach Pearson	-,031	-,248	-,158	,125	-,034	1,000
		Signifikanz (2-seitig)	,941	,553	,708	,769	,937	.
		N	8	8	8	8	8	8
SLE with Nephritis	CreaCl	Korrelation nach Pearson	1,000	,876**	-,730**	-,720**	-,651**	,298
		Signifikanz (2-seitig)	.	,000	,001	,002	,006	,262
		N	16	16	16	16	16	16
	MDRD	Korrelation nach Pearson	,876**	1,000	-,812**	-,799**	-,772**	,124
		Signifikanz (2-seitig)	,000	.	,000	,000	,000	,647
		N	16	16	16	16	16	16
	CysC	Korrelation nach Pearson	-,730**	-,812**	1,000	,920**	,890**	,049
		Signifikanz (2-seitig)	,001	,000	.	,000	,000	,856
		N	16	16	16	16	16	16
	Serum Crea	Korrelation nach Pearson	-,720**	-,799**	,920**	1,000	,970**	-,142
		Signifikanz (2-seitig)	,002	,000	,000	.	,000	,599
		N	16	16	16	16	16	16
	UREA	Korrelation nach Pearson	-,651**	-,772**	,890**	,970**	1,000	-,118
		Signifikanz (2-seitig)	,006	,000	,000	,000	.	,663
		N	16	16	16	16	16	16
	U-Eiw/24h	Korrelation nach Pearson	,298	,124	,049	-,142	-,118	1,000
		Signifikanz (2-seitig)	,262	,647	,856	,599	,663	.
		N	16	16	16	16	16	16

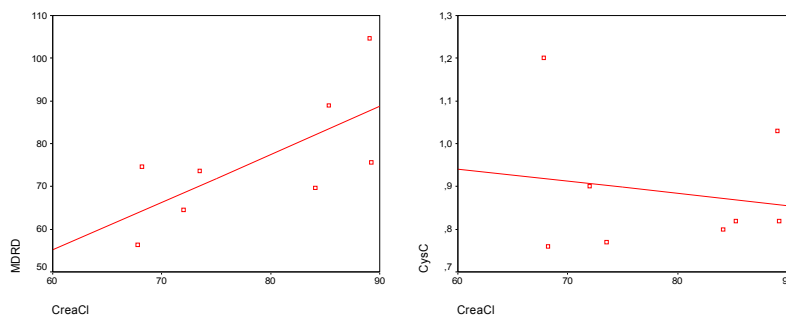
\*\* Die Korrelation ist auf dem Niveau von 0,01 (2-seitig) signifikant.

**Table 14) Correlation analysis regarding renal parameters in both groups**

The next task was to investigate the renal parameters regarding moderate restriction of the kidney function. As described above, “moderate restriction” was defined as  $24hCr_{Cl}$  greater than 60ml/min and lower than 95ml/min. This analysis was performed in the lupus nephritis cohort only, the two patients from group 0 who had a Creatinine Clearance between 90ml/min and 95ml/min were not used for calculation. Again, a list-wise exclusion of missing data was conducted in order to reach a better comparability among the data. The results can be seen in **Table 15**. Eight subjects had a Creatinine Clearance in the given range. Only the MDRD formula showed a noteworthy correlation with  $r=0.69$  and  $p=0.58$

and it fell just short of reaching statistical significance. The other parameters performed distinguishingly worse and Cystatin C along with serum Creatinine had a similar correlation coefficient of about -0.2

For the correlations of MDRD (**Figure 9**) and Cystatin C (**Figure 10**) towards 24hCr<sub>Cl</sub> scatter plots were drawn to graphically emphasize the correlation coefficients of **Table 15**. While **Figure 9** shows a nice distribution and a clearly visible direct proportional correlation, the regression slope for Cystatin C is rather flat and the spots which actually should be all above the 0.9 mark on the y-axis distribute pretty randomly. Another aspect which is shown very well by the scatter plots is the negativity of the correlation coefficient, which is reflected by the falling regression slope, indicating an inversely proportional relation.



**Figure 9) Scatter plot for the correlation of Creatinine Clearance and MDRD**  
**Figure 10) Scatter plot for the correlation of Creatinine Clearance and Cystatin C**

		Korrelationen				
		CreaCl	MDRD	CysC	Serum Crea	UREA
CreaCl	Korrelation nach Pearson	1,000	,691	-,171	-,211	-,289
	Signifikanz (2-seitig)	,	,058	,686	,617	,487
	N	8	8	8	8	3
MDRD	Korrelation nach Pearson	,691	1,000	-,128	-,638	-,605
	Signifikanz (2-seitig)	,058	,	,763	,089	,111
	N	8	8	8	8	3
CysC	Korrelation nach Pearson	-,171	-,128	1,000	,660	,653
	Signifikanz (2-seitig)	,686	,763	,	,075	,075
	N	8	8	8	8	3
Serum Crea	Korrelation nach Pearson	-,211	-,638	,660	1,000	,765*
	Signifikanz (2-seitig)	,617	,089	,075	,	,027
	N	8	8	8	8	3
UREA	Korrelation nach Pearson	-,289	-,606	,658	,765*	1,000
	Signifikanz (2-seitig)	,487	,111	,076	,027	,
	N	8	8	8	8	3

\*. Die Korrelation ist auf dem Niveau von 0,05 (2-seitig) signifikant.

**Table 15) Correlation analysis of renal markers in moderate restriction of the GFR**

The next task was to determine the best marker in the Creatinine blind range, which was defined as a serum Creatinine higher than 0.9 and lower than 1.3. Cystatin C was the only marker showing a significant correlation to the serum Creatinine level in this range with  $r=0.67$  and  $p=0.49$ . However, the correlation of the marker to the 24hCr<sub>Cl</sub> may be of more importance. As can be seen in **Table 16**, none of the markers demonstrated a notably good performance. MDRD had the best correlation with  $r=0.34$  and  $p=0.371$ .

Korrelationen<sup>a</sup>

		CreaCl	MDRD	CysC	Serum Crea	UREA
CreaCl	Korrelation nach Pearson	1,000	,340	-,224	-,272	,073
	Signifikanz (2-seitig)	,	,371	,563	,479	,851
MDRD	Korrelation nach Pearson	,340	1,000	-,114	-,611	-,600
	Signifikanz (2-seitig)	,371	,	,770	,081	,087
CysC	Korrelation nach Pearson	-,224	-,114	1,000	,669*	,573
	Signifikanz (2-seitig)	,563	,770	,	,049	,103
Serum Crea	Korrelation nach Pearson	-,272	-,611	,669*	1,000	,663
	Signifikanz (2-seitig)	,479	,081	,049	,	,050
UREA	Korrelation nach Pearson	,073	-,600	,578	,666	1,000
	Signifikanz (2-seitig)	,851	,087	,103	,050	,

\*. Die Korrelation ist auf dem Niveau von 0,05 (2-seitig) signifikant.

a. Listenweise N=9

**Table 16) Correlation analysis of renal markers in the Creatinine blind range**

### 3.2.2. Renal parameters and body composition

Correlation analysis revealed that renal parameters in the nephritis cohort a higher dependency upon body composition. **Table 17** has been largely reduced in order to provide a better overview. The correlations among the body composition parameters have been deleted as well as the correlations among the nephrological marker, which are shown in **Table 14** anyway.

For Body Mass Index and serum Albumin no statistical significance could be detected in renal marker of either group. Creatinine Clearance showed the best correlation with BMI only reaching a correlation coefficient of  $r=-0.4$  in the nephritis group followed by CysC displaying  $r=0.25$ . The best correlation with serum Albumin was detected in the Cystatin C value of the SLE-only cohort ( $r=-0.47$ ) and in the Creatinine Clearance value in the nephritis cohort ( $r=-0.36$ ). However, both failed reaching significance by far.

Transthyretin shows statistically significant p-values regarding Creatinine Clearance in both groups. Notably, the significance values are very similar with  $p=0.034$  in group 0 and  $p=0.035$  in group 1, respectively, but correlation is directly proportional in the SLE-only cohort while in the nephritis group, the proportionality is inverse. The same phenomenon can be seen in the correlation coefficients of Cystatin C, while MDRD and serum creatinine exhibit the same way of correlation in either group.

**Korrelationen**

Group			BMI	Transthyr	UREA	SerumAlb	TotProt
SLE without Nephritis	CreaCl	Korrelation nach Pearson	-,090	,745*	-,649	,098	-,658
		Signifikanz (2-seitig)	,831	,034	,081	,817	,076
		N	8	8	8	8	8
	MDRD	Korrelation nach Pearson	-,495	-,334	,098	,187	-,114
		Signifikanz (2-seitig)	,212	,418	,817	,657	,788
		N	8	8	8	8	8
	CysC	Korrelation nach Pearson	,060	-,434	,835**	-,472	-,296
		Signifikanz (2-seitig)	,888	,283	,010	,237	,476
		N	8	8	8	8	8
	Serum Crea	Korrelation nach Pearson	,327	,596	-,184	,282	,218
		Signifikanz (2-seitig)	,430	,119	,662	,499	,605
		N	8	8	8	8	8
SLE with Nephritis	CreaCl	Korrelation nach Pearson	-,398	-,529*	-,651**	-,363	-,783**
		Signifikanz (2-seitig)	,127	,035	,006	,168	,000
		N	16	16	16	16	16
	MDRD	Korrelation nach Pearson	-,227	-,421	-,772**	-,253	-,568*
		Signifikanz (2-seitig)	,397	,104	,000	,345	,022
		N	16	16	16	16	16
	CysC	Korrelation nach Pearson	,252	,473	,890**	,068	,389
		Signifikanz (2-seitig)	,347	,064	,000	,804	,137
		N	16	16	16	16	16
	Serum Crea	Korrelation nach Pearson	,228	,486	,970**	,211	,456
		Signifikanz (2-seitig)	,396	,056	,000	,434	,076
		N	16	16	16	16	16

\*. Die Korrelation ist auf dem Niveau von 0,05 (2-seitig) signifikant.

\*\*. Die Korrelation ist auf dem Niveau von 0,01 (2-seitig) signifikant.

**Table 17) Correlation analysis of renal markers regarding body composition**

In the nephritis cohort, all renal parameters correlated with serum Urea high statistically significant, as described above. This time, MDRD and serum Creatinine change their correlation characteristics but in contrast to the phenomenon described for Transthyretin, r-values are very low in the non-nephritis group indicating an accidental change of attitude probably as a consequence of the low numbers analyzed in the SLE-only group.

The serum total protein value correlated high statistically significant with Creatinine Clearance ( $p < 0.000$ ) and statistically significant with MDRD ( $p = 0.22$ ) in the nephritis cohort. Comparing these p-values with the group 0 reveals that  $24hCr_{Cl}$  fell just short reaching significance whereas the p-value of MDRD ( $p = 0.79$ ) was deliberately worse in the other cohort. Serum Creatinine exhibited a low significance level of  $p = 0.76$  in group 1 while its performance was worse in the SLE-only cohort. Cystatin C showed pretty equal p-values in both groups but again, direction of proportionality changed.

Reviewing the data of the SLE-only patients who presented with inadequately high Cystatin C values rose suspicion that elevation of the marker might be associated with low Transthyretin levels. In order to investigate this subject, correlation analysis of the various renal markers in dependency of the Transthyretin level was performed. Data is shown in **Tables 18 and 19**.

**Korrelationen**

		CysC	Transthyr	MDRD	CreaCl
CysC	Korrelation nach Pearson	1,000	,335	-,788**	-,679**
	Signifikanz (2-seitig)	,	,161	,000	,001
	N	19	19	19	19
Transthyr	Korrelation nach Pearson	,335	1,000	-,267	-,154
	Signifikanz (2-seitig)	,161	,	,270	,529
	N	19	19	19	19
MDRD	Korrelation nach Pearson	-,788**	-,267	1,000	,807**
	Signifikanz (2-seitig)	,000	,270	,	,000
	N	19	19	19	19
CreaCl	Korrelation nach Pearson	-,679**	-,154	,807**	1,000
	Signifikanz (2-seitig)	,001	,529	,000	,
	N	19	19	19	19

\*\* . Die Korrelation ist auf dem Niveau von 0,01 (2-seitig) signifikant.

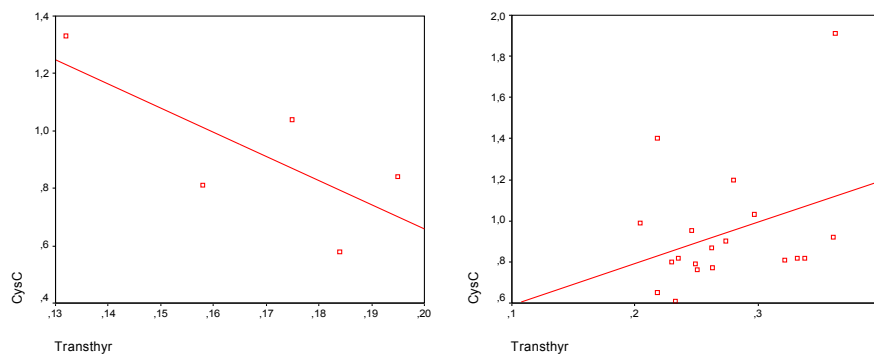
**Table 18) Correlation analysis of renal markers and physiological Transthyretin levels**

**Korrelationen**

		CysC	Transthyr	MDRD	CreaCl
CysC	Korrelation nach Pearson	1,000	-,737	-,468	-,711
	Signifikanz (2-seitig)	,	,156	,427	,178
	N	5	5	5	5
Transthyr	Korrelation nach Pearson	-,737	1,000	,319	,650
	Signifikanz (2-seitig)	,156	,	,601	,235
	N	5	5	5	5
MDRD	Korrelation nach Pearson	-,468	,319	1,000	,878
	Signifikanz (2-seitig)	,427	,601	,	,050
	N	5	5	5	5
CreaCl	Korrelation nach Pearson	-,711	,650	,878	1,000
	Signifikanz (2-seitig)	,178	,235	,050	,
	N	5	5	5	5

**Table 19) Correlation analysis of renal markers and pathological Transthyretin levels**

Analysis shows that the correlation between Cystatin C and Creatinine Clearance shows similar r-values in both cohorts while the significance values vary. When the correlation between Cystatin C and MDRD is investigated, a remarkable difference in both r-value and p-level can be seen. Regarding the performance of the renal markers and Transthyretin, no correlation reaches statistically significant p-values. But in every single parameter the course of correlation changes between the two groups. Cystatin C seems to be unaffected by normal Transthyretin levels with an r-value of 0.34 but the correlation rate is deliberately higher when it comes down to pathological Transthyretin serum values. **Figure 11** shows the correlation of CysC and pathological Transthyretin levels, while in **Figure 12** the performance of Cystatin C towards physiological serum Transthyretin levels is regarded.



**Figure 11) Scatter plot showing the correlation analysis of Transthyretin and Cystatin C in subjects with decreased Transthyretin levels.**

**Figure 12) Scatter plot for the correlation analysis of Transthyretin and Cystatin C in subjects with normal Transthyretin levels.**

### 3.2.3. Renal parameters and inflammation

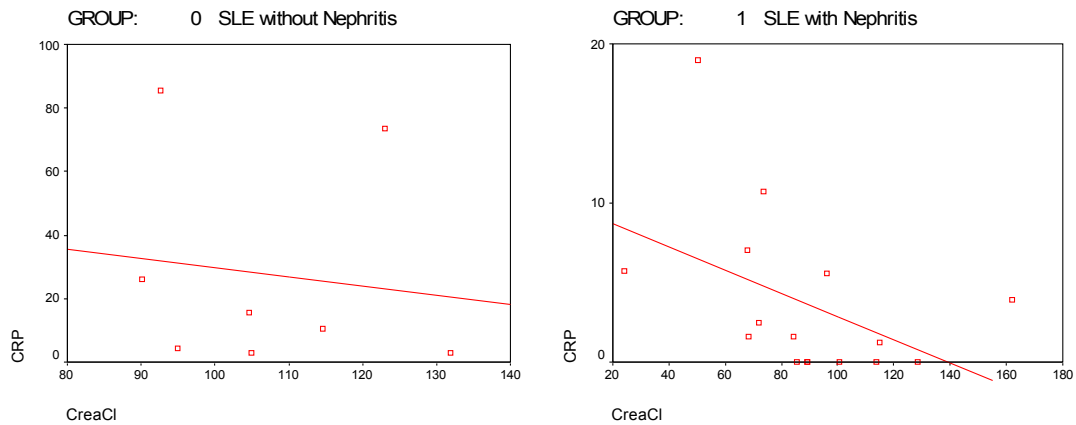
Korrelationen			CRP	WBC	NEU	LY	C3	C4	
Group	SLE without Nephritis	CreaCl	Korrelation nach Pearson	-,132	,274	-,552	,156	,107	-,193
			Signifikanz (2-seitig)	,756	,551	,199	,739	,820	,678
			N	8	7	7	7	7	7
		MDRD	Korrelation nach Pearson	,365	-,044	-,309	-,539	-,254	-,159
			Signifikanz (2-seitig)	,374	,926	,500	,212	,582	,734
			N	8	7	7	7	7	7
	CysC	Korrelation nach Pearson	-,170	-,515	-,330	-,181	,225	,330	
		Signifikanz (2-seitig)	,686	,237	,469	,698	,628	,470	
		N	8	7	7	7	7	7	
	Serum Crea	Korrelation nach Pearson	,048	-,112	-,105	,541	,168	,267	
		Signifikanz (2-seitig)	,910	,812	,823	,210	,719	,562	
		N	8	7	7	7	7	7	
SLE with Nephritis	CreaCl	Korrelation nach Pearson	-,454	,065	,068	,016	-,257	-,269	
		Signifikanz (2-seitig)	,077	,812	,804	,953	,337	,314	
		N	16	16	16	16	16	16	
	MDRD	Korrelation nach Pearson	-,502*	,065	,091	-,025	-,182	-,287	
		Signifikanz (2-seitig)	,048	,810	,737	,927	,500	,282	
		N	16	16	16	16	16	16	
	CysC	Korrelation nach Pearson	,424	-,184	-,181	,032	,181	,115	
		Signifikanz (2-seitig)	,102	,494	,503	,907	,504	,671	
		N	16	16	16	16	16	16	
	Serum Crea	Korrelation nach Pearson	,324	-,007	-,039	,102	,197	,204	
		Signifikanz (2-seitig)	,220	,979	,886	,708	,464	,449	
		N	16	16	16	16	16	16	

\*. Die Korrelation ist auf dem Niveau von 0,05 (2-seitig) signifikant.

**Table 20) Correlation analysis of renal markers and markers reflecting inflammation**

The results of the correlation analysis of renal markers regarding inflammation parameters can be seen in **Table 20**. MDRD - CRP in the Lupus Nephritis cohort is the only correlation reaching a significant result. Creatinine Clearance in the same cohort is on the verge of reaching statistical significance but Cystatin C and serum Creatinine show neither a deliberate correlation rate nor significance level.

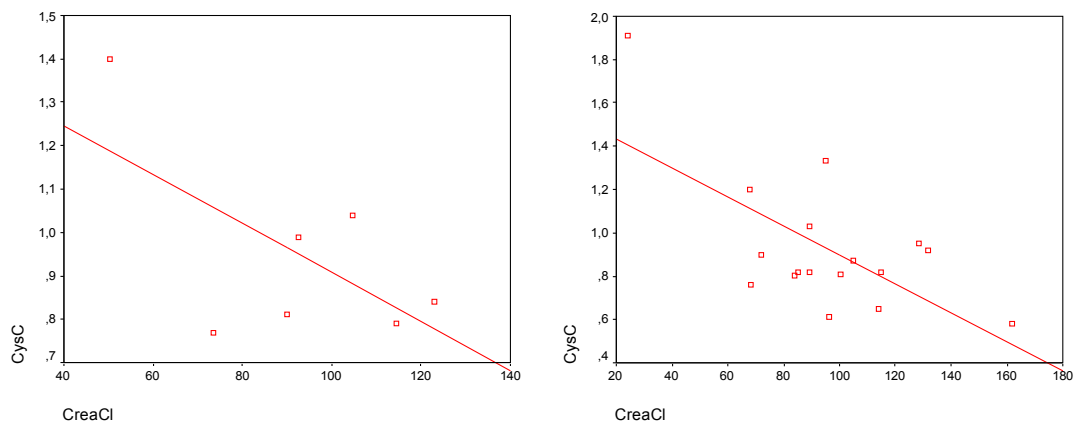
In the SLE only cohort, not a single comparison marks a noteworthy r-value indicating interdependency from all renal markers upon inflammation. This is important, because CRP values in group 0 are significantly higher than in the nephritis group.



**Figure 13) Scatter plot and regression slope for MDRD and CRP in group 0**

**Figure 14) Scatter plot and regression slope for MDRD and CRP in group 1**

In order to graphically compare the correlation analysis, scatter plots were drawn and are shown in the **Figures 13 and 14**. It is crucial to note, that the scaling used in the pictures of group 0 and group 1 are different because the maximum level of the SLE only cohort was significantly higher as mentioned above.



**Figure 15) Scatter plot and regression slope for Cystatin C and CRP with CRP >8mg/l**

**Figure 16) Scatter plot and regression slope for Cystatin C and CRP with CRP <8mg/l**

Because groups differed significantly in CRP and lymphocyte count, it was investigated, if the correlations depend on activity of inflammation and lymphopenia.

In order to do assess the dependence of renal markers upon acute inflammation, the patient collective was divided in a group exhibiting a CRP level greater than 8mg/l and a group presenting with physiological CRP values. Data is shown in **Tables 21 and 22**. Correlation coefficients do not differ in patients suffering from an acute inflammation as compared to subjects who do not exhibit acute inflammation.

Korrelationen

		CreaCl	MDRD	CysC	Serum Crea	UREA
CreaCl	Korrelation nach Pearson	1,000	,942**	-,628	-,830*	-,884**
	Signifikanz (2-seitig)	,	,001	,131	,021	,003
	N	7	7	7	7	7
MDRD	Korrelation nach Pearson	,942**	1,000	-,780*	-,875**	-,882**
	Signifikanz (2-seitig)	,001	,	,038	,010	,003
	N	7	7	7	7	7
CysC	Korrelation nach Pearson	-,628	-,780*	1,000	,911**	,837*
	Signifikanz (2-seitig)	,131	,038	,	,004	,013
	N	7	7	7	7	7
Serum Crea	Korrelation nach Pearson	-,830*	-,875**	,911**	1,000	,904**
	Signifikanz (2-seitig)	,021	,010	,004	,	,005
	N	7	7	7	7	7
UREA	Korrelation nach Pearson	-,884**	-,882**	,837*	,904**	1,000
	Signifikanz (2-seitig)	,008	,009	,019	,005	,
	N	7	7	7	7	7

\*\* Die Korrelation ist auf dem Niveau von 0,01 (2-seitig) signifikant.

\* Die Korrelation ist auf dem Niveau von 0,05 (2-seitig) signifikant.

**Table 21) Correlation analysis of renal markers in patients with CRP>8**

Korrelationen

		CreaCl	MDRD	CysC	Serum Crea	UREA
CreaCl	Korrelation nach Pearson	1,000	,812**	-,649**	-,687**	-,635**
	Signifikanz (2-seitig)	,	,000	,005	,002	,003
	N	17	17	17	17	17
MDRD	Korrelation nach Pearson	,812**	1,000	-,676**	-,814**	-,691**
	Signifikanz (2-seitig)	,000	,	,003	,000	,002
	N	17	17	17	17	17
CysC	Korrelation nach Pearson	-,649**	-,676**	1,000	,818**	,883**
	Signifikanz (2-seitig)	,005	,003	,	,000	,003
	N	17	17	17	17	17
Serum Crea	Korrelation nach Pearson	-,687**	-,814**	,818**	1,000	,883**
	Signifikanz (2-seitig)	,002	,000	,000	,	,003
	N	17	17	17	17	17
UREA	Korrelation nach Pearson	-,635**	-,691**	,888**	,889**	1,000
	Signifikanz (2-seitig)	,006	,002	,000	,000	,
	N	17	17	17	17	17

\*\* Die Korrelation ist auf dem Niveau von 0,01 (2-seitig) signifikant.

**Table 22) Correlation analysis of renal markers in patients with CRP<8**

Finally, the patients were divided into two groups whereas one cohort had lymphopenia and the other showed normal lymphocyte count. Data is depicted in the **Tables 23 and 24**. Patients exhibit by far worse r-values if they suffer from lymphopenia. As can be seen in **Table 23**, not a single correlation between Cystatin C and any other marker reflecting renal function reaches statistical significance if absolute lymphocyte count is decreased while the r-values deliberately ameliorate if lymphocyte count is in the physiological range.

Korrelationen

		CreaCl	MDRD	CysC	Serum Crea	UREA
CreaCl	Korrelation nach Pearson	1,000	,857**	-,388	-,825*	-,595
	Signifikanz (2-seitig)	,	,007	,343	,012	,123
	N	8	8	8	8	3
MDRD	Korrelation nach Pearson	,857**	1,000	-,315	-,979**	-,221
	Signifikanz (2-seitig)	,007	,	,447	,000	,593
	N	8	8	8	8	3
CysC	Korrelation nach Pearson	-,388	-,315	1,000	,164	,683
	Signifikanz (2-seitig)	,343	,447	,	,698	,063
	N	8	8	8	8	3
Serum Crea	Korrelation nach Pearson	-,825*	-,979**	,164	1,000	,113
	Signifikanz (2-seitig)	,012	,000	,698	,	,782
	N	8	8	8	8	3
UREA	Korrelation nach Pearson	-,595	-,221	,686	,118	1,000
	Signifikanz (2-seitig)	,120	,599	,060	,782	,
	N	8	8	8	8	3

\*\* Die Korrelation ist auf dem Niveau von 0,01 (2-seitig) signifikant.

\* Die Korrelation ist auf dem Niveau von 0,05 (2-seitig) signifikant.

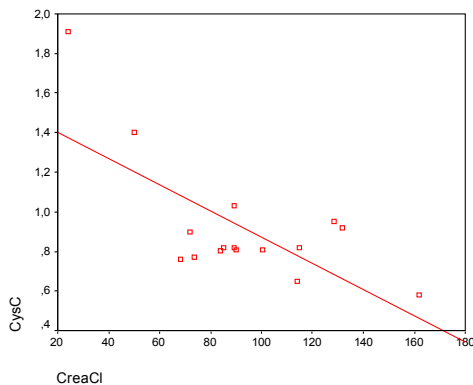
**Table 23) Correlation analysis of renal markers in patients with lymphopenia (LY<1.0G/l)**

		CreaCl	MDRD	CysC	Serum Crea	UREA
CreaCl	Korrelation nach Pearson	1,000	,847**	-,691**	-,686**	-,643**
	Signifikanz (2-seitig)	,	,000	,003	,003	,007
	N	16	16	16	16	13
MDRD	Korrelation nach Pearson	,847**	1,000	-,795**	-,809**	-,787**
	Signifikanz (2-seitig)	,000	,	,000	,000	,000
	N	16	16	16	16	13
CysC	Korrelation nach Pearson	-,691**	-,795**	1,000	,941**	,915**
	Signifikanz (2-seitig)	,003	,000	,	,000	,000
	N	16	16	16	16	13
Serum Crea	Korrelation nach Pearson	-,686**	-,809**	,941**	1,000	,967**
	Signifikanz (2-seitig)	,003	,000	,000	,	,000
	N	16	16	16	16	13
UREA	Korrelation nach Pearson	-,649**	-,787**	,915**	,967**	1,000
	Signifikanz (2-seitig)	,007	,000	,000	,000	,
	N	16	16	16	16	13

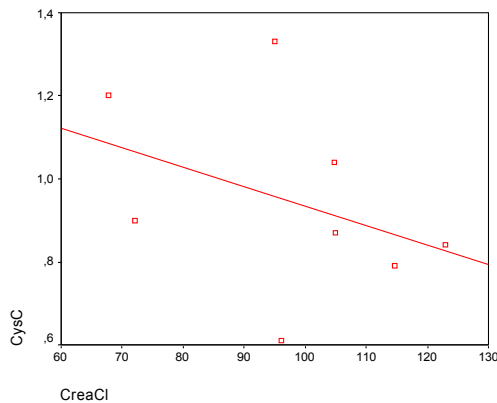
\*\* Die Korrelation ist auf dem Niveau von 0,01 (2-seitig) signifikant.

**Table 24) Correlation analysis of renal markers in patients without lymphopenia.**

In order to graphically emphasize this result, scatter plots for the correlation of 24hCr<sub>Cl</sub> and Cystatin C were drawn and they are shown in the **Figures 17 and 18**. It can be seen that in **Figure 17** there is a clear negative correlation between the investigated parameters and the regression slope is steep. In the lymphopenia cohort, the distribution pattern is random and the regression slope is clearly flatter than in the picture shown above.



**Figure 17) Scatter plot and regression slope for Creatinine Clearance - Cystatin C in patients with normal absolute lymphocyte count**



**Figure 18) Scatter plot and regression slope for Creatinine Clearance and Cystatin C in patients with lymphopenia**

### 3.2.1. Renal Markers and Thyroid Function

As mentioned above, the relation between Cystatin C and Creatinine Clearance was inconsistent which raised suspicion that a certain factor might influence the serum level of the investigated markers. The fact that Transthyretin might contribute to the confounding suggested thyroid function as the most possible candidate for Transthyretin is a thyroid hormone binding serum protein. Analysis was conducted retrospectively and the results are shown in **Tables 25 and 26**.

Statistiken			TSH	FT3	FT4
SLE without Nephritis	N	Gültig	6	6	6
		Fehlend	2	2	2
	Mittelwert	1,3533	5,7000	15,5833	
	Median	1,2200	4,5500	14,6000	
	Varianz	1,4764	9,9880	6,1097	
	Minimum	,03	4,00	12,90	
	Maximum	3,04	12,10	19,40	
SLE with Nephritis	N	Gültig	11	11	11
		Fehlend	5	5	5
	Mittelwert	1,5945	4,4000	16,8545	
	Median	1,5000	4,3000	16,3000	
	Varianz	,5829	,5480	5,8587	
	Minimum	,35	3,30	13,50	
	Maximum	3,17	5,80	20,50	

**Table 25) Descriptive analysis of thyroid hormones**

Prior to correlation analysis, the most important parameters of descriptive analysis were investigated. In seventeen cases the thyroid hormone levels could be obtained. All mean and median values were in the normal range and only the patient who presented with M. Base-dow showed pathologic values. This may also explain the trend towards lower TSH and higher FT3 levels in SLE only cohort.

Korrelationen<sup>a,b</sup>

Group			CreaCl	MDRD	CysC	TSH	FT3	FT4
SLE without Nephritis	CreaCl	Korrelation nach Pearson	1,000	-,088	-,588	-,178	-,415	-,111
		Signifikanz (2-seitig)	,	,868	,220	,736	,414	,835
	MDRD	Korrelation nach Pearson	-,088	1,000	,222	-,627	,466	,936*
		Signifikanz (2-seitig)	,868	,	,673	,183	,352	,013
	CysC	Korrelation nach Pearson	-,588	,222	1,000	-,170	,909*	,438
		Signifikanz (2-seitig)	,220	,673	,	,748	,012	,349
	TSH	Korrelation nach Pearson	-,178	-,627	-,170	1,000	-,475	-,735
Signifikanz (2-seitig)		,736	,183	,748	,	,341	,059	
FT3	Korrelation nach Pearson	-,415	,466	,909*	-,475	1,000	,744	
	Signifikanz (2-seitig)	,414	,352	,012	,341	,	,030	
FT4	Korrelation nach Pearson	-,111	,906*	,468	-,795	,744	1,000	
	Signifikanz (2-seitig)	,835	,013	,349	,059	,090	,	
SLE with Nephritis	CreaCl	Korrelation nach Pearson	1,000	,902**	-,740**	,252	-,054	-,029
		Signifikanz (2-seitig)	,	,000	,009	,455	,874	,933
	MDRD	Korrelation nach Pearson	,902**	1,000	-,886**	,249	-,023	,131
		Signifikanz (2-seitig)	,000	,	,000	,460	,947	,636
	CysC	Korrelation nach Pearson	-,740**	-,886**	1,000	-,124	,006	-,322
		Signifikanz (2-seitig)	,009	,000	,	,715	,986	,335
	TSH	Korrelation nach Pearson	,252	,249	-,124	1,000	-,495	-,635*
Signifikanz (2-seitig)		,455	,460	,715	,	,121	,036	
FT3	Korrelation nach Pearson	-,054	-,023	,006	-,495	1,000	,249	
	Signifikanz (2-seitig)	,874	,947	,986	,121	,	,459	
FT4	Korrelation nach Pearson	-,029	,161	-,322	-,635*	,249	1,000	
	Signifikanz (2-seitig)	,933	,636	,335	,036	,459	,	

\*. Die Korrelation ist auf dem Niveau von 0,05 (2-seitig) signifikant.

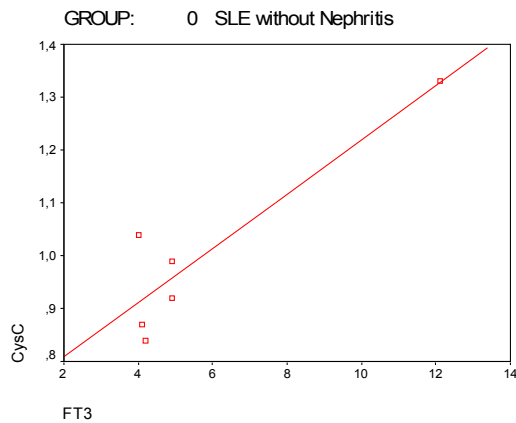
\*\* Die Korrelation ist auf dem Niveau von 0,01 (2-seitig) signifikant.

a. Listenweise N=6

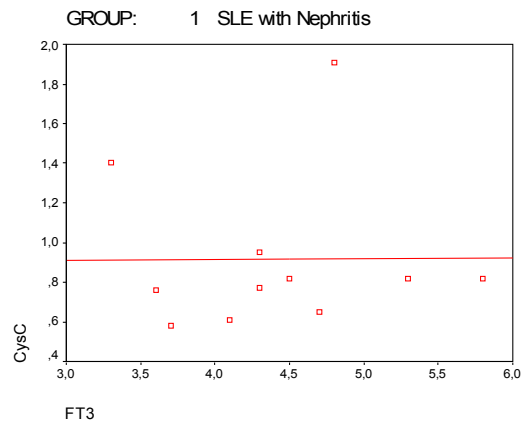
b. Listenweise N=11

**Table 26) Correlation analysis of renal function and thyroid markers**

**Table 26** shows the results of the correlation analysis. In the SLE only cohort, MDRD and CysC show a very poor correlation towards Creatinine Clearance and a statistically significant correlation with ft3 (Cystatin C) and ft4 (MDRD), while the correlations in the nephritis cohort are completely the other way round.



**Figure 19) Scatter plot and regression slope for ft3 - CysC in group 0**



**Figure 20) Scatter plot and regression slope for ft3 - CysC in group 1**

In order to provide help explaining these results, scatter plots were drawn. It must be noted that there is a deliberate difference in the scaling of these two graphs because of the outstanding high fT3 level of the Graves' disease patient. But still, it can be seen in **Figure 19** that the three patients with elevated or borderline Cystatin C value do have normal fT3 levels. The regression slope in **Figure 18** is merely flat but also two runaways influence this figure distinguishingly and they refer to two patients with very bad Creatinine Clearance and low MDRD.

## 4. Discussion

### 4.1. Renal Parameters

The results show a trend towards worse renal function in the nephritis cohort which is reflected best by the low p-value for 24hCr<sub>Cl</sub>. MDRD and Cystatin C show a similar performance in their significance levels, but it is crucial to mention that mean and median Cystatin C values were surprisingly higher in the SLE-only group. This is confusing and hints that CysC levels are being influenced by factors other than the GFR.

In total, three subjects suffering from SLE without nephritis showed elevated Cystatin C values with both MDRD and Creatinine Clearance being low, but still in the physiologic range. This also indicates either influences upon the Cystatin C level other than renal impairment or elevation of the investigated marker very early in the course of the disease. The follow-up of these patients is being watched and will be reported. In opposite to that, six patients suffering from Lupus Nephritis presented normal Cystatin C levels despite a pathological Creatinine Clearance which strongly indicates influencing factors but however, neither body composition nor inflammation sufficiently explain these figures as described below.

Correlation analysis, shown in **Table 14**, revealed a great correlation coefficient for CysC and MDRD in the nephritis cohort, but surprisingly, r-values in the SLE-only group were poor. In fact, serum Urea was the only marker showing a comparable correlation towards 24hCr<sub>Cl</sub> in both groups. As a consequence, a confounding factor must be assumed which leads to this disturbance. A difference in the quality of urine collection between the cohorts can not explain this dilemma, because correlations of serum-only markers (e.g. CysC - serum Crea) also exhibit that difference and the sera were analyzed in the same lab. In fact, this serves as evidence that the confounder has to be looked for in the serum.

The results which were obtained analyzing the performances of the renal markers in the Creatinine blind range and in the scope defined as “moderate restriction of the kidney function” are most probably not affected by the confounder because only patients of group 1 were investigated. Correlation analysis for moderate restriction revealed that MDRD formula was the only marker reaching a satisfying r-value, while Cystatin C, serum Urea and serum Creatinine show disappointing results. Regarding the Creatinine blind range, all correlation coefficients were low. The importance of these figures must not be overestimated. Analysis included only nine subjects in the Creatinine blind range statistic and eight patients in the other cohort, respectively. But still it should be noted that Cystatin C does not exhibit an advantage of MDRD in borderline kidney function as was expected.

## **4.2. Body composition**

Analysis revealed a good comparability of the groups regarding their body composition. No mean or median value of any single marker was in the pathologic range which is to say that Systemic Lupus Erythematosus does not influence Body Mass Index or the other parameters per se. Serum total protein and albumin levels were of special interest because they are altered in nephrotic syndrome along with serum lipids. Only a single patient in the nephritis cohort exhibited a constellation which can be interpreted as a nephrotic syndrome. This patient suffers from WHO grade IV Lupus Nephritis and exhibited a borderline Cystatin C value despite a Creatinine Clearance of 128ml/min and MDRD.

Correlation analysis indicated a slight influence of the body composition on the renal markers. However, Transthyretin shows a puzzling relation to 24hCr<sub>Cl</sub> and Cystatin C. Both markers exhibit quite high r- and p-values in both groups but the sign of the correlation coefficient is different among the two cohorts. Even more surprising, the correlations of Transthyretin regarding MDRD and serum Creatinine are also high but still, the sign remains the same. Most probably, this is a chance finding and this hardly explicable situation is a tribute to the low number of patients included.

At first sight it seemed as if low Transthyretin level was associated with higher Cystatin C values leading to an underestimation of renal function because none of the patients with low Transthyretin and high CysC values had a pathological renal function when MDRD and 24hCr<sub>Cl</sub> were regarded. However, correlation analysis was difficult to interpret, because only five patients showed a pathological serum Transthyretin level. The correlation

coefficients for CysC and MDRD towards  $24\text{hCr}_{\text{Cl}}$  were quite similar in both groups leading to the conclusion that low serum Transthyretin does not affect the quality of these parameters. One must not overestimate the differences regarding the p-value because it is influenced by the number of subjects used in analysis to a great extent. Consecutively, the big difference in  $n$  can serve as an explanation for the inadequacy in the p-value but moreover, body composition is not to be held responsible for the bad correlation of the renal parameters, as mentioned above.

### **4.3. Renal markers and Inflammation**

Inflammation was the only subject in which the patient collectives differed to a statistically significant extent. But as many different studies have shown, Cystatin C level is not influenced by inflammation. The correlation rate for MDRD - CRP in the nephritis cohort was the only one reaching statistical significance. However, the fact that CRP and absolute lymphocyte count differed significantly among the cohorts raised suspicion that they might contribute to the disturbance mentioned above. **Tables 21 and 22** give proof that CRP is not the confounding factor in this trial because r-values are pretty similar in both cohorts. Analysis revealed that lymphopenia is associated with a derangement in the correlation of Cystatin C and other renal parameters. Unfortunately, this can not serve as the ultimate explanation because in the SLE-only cohort correlations other than those with Cystatin C were off the top but still, from this data, an important influence can not be denied.

### **4.5. Renal parameters and Thyroid Function**

The search for other parameters which could probably influence this disturbance raised suspicion that the confounding factor could be the function of the thyroid gland. The rationale was the fact that Transthyretin, which was the first suspect, is a thyroid hormone binding protein, comparable to Thyreoglobulin and therefore, it is associated to thyroid hormone levels. Unfortunately, the studies indicating the dependence of serum Cystatin C level upon thyroid function were found way after the collection of the patients had been performed. This implicates that thyroid hormones were attained *a posteriori*, but still, they were available in seventeen patients. The results must be interpreted cautiously. The significant correlation of fT3 and fT4 with MDRD and Cystatin C in the SLE-only cohort would excellently explain the bad correlation of these parameters with  $24\text{hCr}_{\text{Cl}}$ . But why does the

r-value change so dramatically among the two cohorts? A possible factor might be that the Mb. Basedow patient leads to a runaway-bias of the results. This is to say, it pretends a high r-value by markedly overestimating the correlation. This kind of thyroid hormone level which is outstandingly different from the values of the other patients is present only in the non-nephritis group and deliberately influences the result. This theory is fortified by the scatter plots.

But the patient who suffers from Graves' disease still is very important for this study. Recent data indicated that Cystatin C is overestimated in a hyperthyroid state which is confirmed by this patients' data. Creatinine Clearance (95.0ml/min) and MDRD (101.07ml/min) are in the normal range but Cystatin C is massively elevated with 1.33mg/l while Thyroid Stimulating Hormone is reduced with 0.03mU/l.

#### **4.6. Conclusion**

Despite several limitations, this study has provided some additional information regarding the clinical use of Cystatin C in Lupus Nephritis. The main problem was the low number of patients included which was due to the fact that SLE is a rare disease and twenty-four hour Creatinine Clearance could only be obtained in a small number of patients visiting the day care units. Another problem was that Creatinine Clearance had to be used as the reference method of assessing the GFR but as described in the introduction, this method is accompanied by multiple problems which often lead to an incorrect estimation of the renal function.

But this trial also brought some interesting new insights in the value of Cystatin C in estimating the renal function. Firstly, Cystatin C seems to be influenced to a great extent by pathologies of the thyroid gland which was reported by others, but also confirmed in this trial by the Mb. Basedow patient. Thyroid dysfunction was present in seven patients indicating that it is a common co-existing problem in SLE patients.

Furthermore, this trial suggests that lymphopenia being an additional and not yet described factor which influences the level of serum Cystatin C. The reason for this probably lies in the function of CysC as a proteinase inhibitor. Unfortunately, no data exists defining the role of SLE-lymphocytes regarding cathepsins and/or Cystatin C production. In order to confirm or to negative this theory, a study with a larger cohort would be needed.

As a consequence of this trial, estimating the renal function in Lupus and Lupus Nephritis remains a difficult task and Cystatin C does not greatly improve the pre-existing dilemma. It seems to deliberately depend on thyroid function and its serum level is apparently influenced by lymphopenia which does not make Cystatin C a very favorable marker of renal function, since both entities are common problems featured in Systemic Lupus Erythematosus. In order to diagnose or to estimate the course of this critical disease, renal parameters are best regarded in total.

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