KRISTA KARSTILA

Incidence and prognosis of renal diseases and measurement of renal function in patients with rheumatoid arthritis

ACADEMIC DISSERTATION
To be presented, with the permission of the Faculty of Medicine of the University of Tampere, for public discussion in the Small Auditorium of Building K, Medical School of the University of Tampere, Teiskontie 35, Tampere, on May 8th, 2009, at 12 o’clock.

UNIVERSITY OF TAMPERE
To my family
ABSTRACT

Background. Rheumatoid arthritis (RA) is a systemic inflammatory disease affecting abov all the joints, but clinical signs of renal disease (proteinuria, hematuria and chronic renal failure, CRF) are also often seen. Predominant RA-related underlying causes for here are mesangial glomerulonephritis (MesGN), amyloidosis, and drugs, and optimal medical treatment requires the clinician to be aware of the patient’s precise glomerular filtration rate (GFR). Estimation of renal function has traditionally been based on measurement of serum creatinine, although this is known to be an insensitive measure of GFR. The treatment of RA has evolved rapidly over the last two decades, and intensive combination disease-modifying antirheumatic drug (DMARD) therapy is nowadays a widely accepted strategy. Knowledge of the renal safety of this approach is, however, scant.

Aims. The present purpose was to establish the long-term outcome of abnormal clinical renal findings and RA-related renal diseases in a 13-year follow-up of a cross-sectional population-based cohort of RA patients. The incidence of new clinical renal findings was also evaluated in this advanced RA population as well as in a population of early RA patients (11-year follow-up study in the FIN-RACo trial). A further aim was to assess the diagnostic accuracy of six measures of GFR in RA patients and to evaluate the renal safety of initial intensive treatment with combination DMARDs in early RA.

Patients and methods. Subjects for studies I and II were selected from a population-based cohort of RA patients living in Tampere in 1987. In the cross-sectional study conducted in 1988, clinical renal findings were recorded in 103 of these patients (nephropathy patients, NP) including 34 with proteinuria, 54 with isolated hematuria and 15 with isolated CRF. Also 17 patients with MesGN were found (study II). Matched controls (n=102) were selected from among RA patients yielding no clinical renal findings. In 2003, a follow-up study was made of these 205 patients, of whom 72 attended and 133 were assessed from hospital records. In study III, 64 RA patients were enrolled in conjunction with a routine follow-up appointment in the outpatient ward. The diagnostic accuracy of plasma creatinine, cystatin C, urea, creatinine clearance, Cockcroft-Gault (CG) formula and Modification of Diet in Renal Disease (MDRD) formula were studied using the plasma clearance of $^{51}$Cr-EDTA as reference. The population in study IV comprised 195 DMARD- and glucocorticoid-naïve patients with recent-onset RA (FIN-RACo Trial). The cumulative 11-year incidence of repeated clinical nephropathy findings and also the renal safety of initial intensive combination DMARD treatment (COMBI) compared to traditional single DMARD therapy (SINGLE) were evaluated.

Results. Proteinuria persisted in the median 13-year follow-up in 19 out of 34 (56%) advanced RA patients evincing proteinuria in 1988. Serum creatinine exceeded 200 µmol/l in 35 % of the patients in this original group and renal replacement therapy was given to 26 %. Isolated hematuria continued in the follow-up in 28 % of the original 54 hematuria patients, serum creatinine exceeding 200 µmol/l in 8 % and dialysis therapy being given to one patient. In the isolated CRF group (n=15) the finding continued in 53 %, but in all cases serum creatinine remained below 200 µmol/l. New clinical renal findings, mostly mild in character, were detected in 28% of the 102 control patients. The clinical
prognosis of renal amyloidosis in the NP group proved poor; serum creatinine exceeded 200 µmol/l in 15 out of the 20 patients with definite or probable renal amyloidosis, and dialysis therapy was given to 9 out of these 20 patients. In RA patients with MesGN the clinical renal findings normalized in 6 out of the 17 patients and MesGN was found as the sole reason for CRF in none.

In the early-RA population the cumulative occurrences of repeated proteinuria during the 11-year follow-up were 4.8% in the COMBI group and 5.3% in the SINGLE group. of repeated hematuria in 14.1% and 22.1% and of repeated findings of estimated GFR < 60 ml/min/1.73m$^2$ (CG) in 11.9% and 10.5%, respectively. No significant differences were detected in the cumulative incidences of the findings.

Creatinine clearance and the CG formula proved superior in identifying GFR < 90ml/min/1.73m$^2$ in RA patients as against plasma creatinine, cystatin C or urea. Plasma creatinine measurement left 42% of GFR < 90ml/min/1.73m$^2$ undetected, while the corresponding figure for the CG formula was 12.

**Conclusions.** All clinical renal findings recorded in 1988 continued quite frequently in the 13-year follow-up, but isolated proteinuria, proteinuria combined with hematuria and CRF were found to adumbrate an unfavourable clinical outcome. Isolated hematuria and isolated CRF, again, were associated with an evidently better prognosis with good preservation of renal function and infrequent need for dialysis therapy. MesGN as a sole finding did not lead to renal functional impairment, whereas renal amyloidosis remained a serious condition in advanced RA patients, being treated mostly with traditional DMARDs. Use of the CG formula or creatinine clearance is recommended for the estimation of GFR in RA patients instead of using solely creatinine, cystatin C or urea. Finally, the initial intensive combination DMARD therapy applied in early RA did not increase the cumulative incidence of clinical renal findings compared to traditional therapy with a single DMARD.
TIIVISTELMÄ


8\%:lla, 8\%:lla koko ryhmän kreatiniinitaso ylitti 200 µmol/l ja yhdelle potilaista käynnistettiin dialyysihoido seurannassa. CRF jatkui 53 \%:lla pelkän CRF-ryhmän potilaista (n=15), mutta kreatiniinitaso ei ylittänyt 200 µmol/l kenelläkään. Verrokkiryhmässä (n=102), joilla vuonna 1988 ei ollut munuaislöydöksiä, todettiin seurannassa 28 \%:lla uusia, pääosin lieviä nefrologisia löydöksiä. Munuaisamyloidoosin kliinin prognoosi todettiin huonoksi NP-ryhmässä; 9 potilasta 20:stä, joilla oli varma tai todennäköinen munuaisamyloidoosi, tarvitsi dialyysihoidoa ja 15/20 potilaan kreatiniini ylitti tason 200 µmol/l. Potilailla, joilla todettiin MesGN, kliiniset löydökset normalisoituvat 6 potilaalla 20:stä ja löyös ei yksinään johtanut munuaisten vajaatoimintaan. Varhaisessa nivelreumapopulaatiossa ei todettu tilastollista eroa toistuvien munuaislöydöksen kemumatiivisissä ilmaantuvuksissa hoitostrategioiden välillä. Ilmaantuvuudet olivat 11v seurannassa COMBI- ja SINGLE-ryhmissä: valkuaisvirtsaisuus 4.8 \% ja 5.3 \%, verivirtsaisuus 14.1 \% ja 22.1 \%, CG-kaavalla arvioitu GFR alle 60 ml/min/1.73m$^2$ 11.9 \% ja 10.5 \%.

Kreatiniinin puhdistuma ja CG-kaava tunnistivat plasman kreatiniini-, kystatiini C- tai urea-mittausta paremmin nivelreumapotilaat, joilla GFR oli alle 90 ml/min/1.73m$^2$. Kreatiniini-mittaus jätti huomioimatta 42 \% potilaista, joilla GFR oli alle 90 ml/min/1.73m$^2$, kun taas CG-kaavaa käytettäessä lukema oli 12 \%.

## CONTENTS

ABSTRACT ........................................................................................................................................... 5  
TIIVISTELMÄ ....................................................................................................................................... 7  
CONTENTS .......................................................................................................................................... 9  
ABBREVIATIONS: ................................................................................................................................. 11  
LIST OF ORIGINAL COMMUNICATIONS .............................................................................................. 12  
INTRODUCTION .................................................................................................................................... 13  
REVIEW OF THE LITERATURE ............................................................................................................... 15  

1. Abnormal clinical renal findings in patients with RA ........................................................................... 15  
   1.1 Proteinuria .................................................................................................................................. 15  
      1.1.1 Prevalence and incidence of proteinuria in RA patients and in the normal population .... 16  
      1.1.2. Associations of proteinuria in patients with RA ................................................................. 17  
   1.2 Hematuria .................................................................................................................................... 18  
      1.2.1 Prevalence and incidence of hematuria in RA patients and in the normal population ...... 19  
      1.2.2. Associations of hematuria in patients with RA ................................................................. 20  
   1.3 Chronic renal failure ..................................................................................................................... 21  
      1.3.1 Prevalence and incidence of chronic renal failure in RA patients and in the normal population......................................................................................................................... 22  
      1.3.2 Associations of chronic renal failure in patients with RA .................................................. 23  

2. RA-associated renal diseases ................................................................................................................ 24  
   2.1 Renal AA-amyloidosis .................................................................................................................. 25  
   2.2 Mesangial glomerulonephritis (MesGN) ..................................................................................... 29  
   2.3 Other diseases ............................................................................................................................. 31  

3. Renal diseases related to DMARDs and NSAIDs .................................................................................. 32  
   3.1 Gold salts and D-penicillamine ..................................................................................................... 32  
   3.2 Cyclosporine .................................................................................................................................. 33  
   3.3 Other disease-modifying antirheumatic drugs (DMARDs) .......................................................... 34  
   3.4 Non-steroidal anti-inflammatory drugs (NSAIDs) ...................................................................... 36  

4. Measurement of renal function in RA patients ..................................................................................... 37  
   4.1 Chronic kidney disease and glomerular filtration rate ................................................................. 37  
   4.2 The significance of estimating glomerular filtration rate in RA patients ..................................... 38  
   4.3 Measurement of exogenous substances ....................................................................................... 39  
   4.4 Plasma creatinine and creatinine clearance .................................................................................. 39  
   4.5 Creatinine-based prediction equations ......................................................................................... 41  
   4.6 Plasma cystatin C ......................................................................................................................... 43  
   4.7 Plasma urea .................................................................................................................................. 45  

AIMS OF THE STUDY ............................................................................................................................ 47  
STUDY POPULATIONS AND METHODS .............................................................................................. 48  

1. Populations ........................................................................................................................................ 49  
   1.1 Study I ....................................................................................................................................... 49  
   1.2 Study II .................................................................................................................................... 50  
   1.3 Study III ................................................................................................................................... 50
ABBREVIATIONS:

AA  amyloid A
BMI  body mass index
CKD  chronic kidney disease
CRF  chronic renal failure
CRP  C reactive protein
CG  Cockcroft-Gault
COMBI combination DMARD therapy
COX  cyclo-oxygenase
DMARD disease-modifying antirheumatic drug
DPA  D-penicillamine
eGFR  estimated glomerular filtration rate
ESR  erythrocyte sedimentation rate
FIN-RACo  Finnish Rheumatoid Arthritis Combination therapy
GFR  glomerular filtration rate
IQR  interquartile range
K/DOQI  Kidney Disease Outcomes Quality Initiative
MesGN  mesangial glomerulonephritis
MDRD  Modification of Diet in Renal Disease
NP  nephropathy patients
p-ANCA  perinuclear antineutrophil cytoplasmic antibodies
NSAID  non-steroidal anti-inflammatory drug
RA  rheumatoid arthritis
REKO  tuoreen nivelreuman yhdistelmähoito
RF  rheumatoid factor
SAA  serum amyloid A protein
SINGLE single-DMARD therapy
TNF-α tumor necrosis factor α
LIST OF ORIGINAL COMMUNICATIONS

This thesis is based on the following original communications, referred to in the text by the Roman numerals I-IV:


The original publications are reprinted with the permission of the copyright holders.
INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease of unknown etiology characterized by polyarticular joint inflammation leading to joint destruction, functional disability, and decreased life expectancy (Pincus and Callahan 1989). Extra-articular disease manifestations such as rheumatoid nodules, secondary Sjögren’s syndrome and pulmonary fibrosis occur in about 40% of patients (Turesson et al. 2003). Some of the extra-articular manifestations can also affect the kidneys, for example amyloidosis, mesangial glomerulonephritis (MesGN) and vasculitis (Pollak et al. 1962; Sellars et al. 1983; Boers et al. 1987; Helin et al. 1995; Korpela et al. 1997).

Abnormal clinical renal findings such as proteinuria, hematuria or chronic renal failure (CRF) are often seen in patients with RA (Sørensen 1962; Richards et al. 1988; Cantagrel et al. 1990; Korpela 1993; Koseki et al. 2001), but reports on the incidence of these findings in early RA patients are scant (Koseki et al. 2001). Especially CRF has been variously defined in the literature and most studies have used divergent cut-off points for serum creatinine as a defining method. However, the use of the serum concentration of creatinine as an index for the glomerular filtration rate (GFR) is problematic, especially in RA patients, by reason of the insensitivity of the measurement (Nived et al. 1983; Boers et al. 1988; Perrone et al. 1992). The predominant diagnoses related to clinical renal findings have been amyloidosis, MesGN and also drug-related renal disease (Sellars et al. 1983; Hordon et al. 1984; Helin et al. 1986; Hazenberg and van Rijswijk 1994).

The management of RA has evolved rapidly over the last few decades. From the 1960s to the 1980s RA patients were traditionally treated with single disease-modifying antirheumatic drug (DMARD) therapy applying a “pyramidal” strategy. Treatment commenced with symptom-relieving
drugs, and DMARDs were subsequently introduced one by one, starting with the least toxic (Copeman 1964). In the 1980s a “sawtooth” strategy was introduced advocating early, continual and serial use of DMARDs (Fries 1990). The observation that the efficacy of DMARD monotherapy in RA tends to diminish with time (Wolfe et al. 1990) led to the use of combinations of DMARDs, which have proved superior to monotherapy in both early and longstanding RA (Tugwell et al. 1995; Boers et al. 1997; Möttönen et al. 1999; Landewe et al. 2002). In the last decade the combinations have increasingly included biologicals, especially anti-tumor necrosis factor alpha (TNF-α) agents, further increasing the efficacy and range of treatments (Weinblatt et al. 1999; Breedveld et al. 2004; Klareskog et al. 2004). The initiation of combination therapy has not been associated with any increase in side-effects (O'Dell et al. 1996; Möttönen et al. 1999; Landewe et al. 2002), but renal safety has not been specifically evaluated.

The purpose of the present series was to evaluate the long-term renal prognosis of amyloidosis, MesGN and clinical renal findings in an advanced RA population, and to establish the incidence of abnormal clinical renal findings in advanced and early RA populations. The influence of treatment strategy on the incidence of abnormal clinical renal findings was assessed in the early RA population, paying special attention to the renal safety of modern combination DMARD therapies. The diagnostic accuracy of different means of assessing GFR in RA patients was evaluated.
REVIEW OF THE LITERATURE

1. Abnormal clinical renal findings in patients with RA

Clinical signs of renal disease (proteinuria, hematuria and impaired renal function) are often seen in patients with RA (Sørensen 1964; Dieppe et al. 1976; Boers et al. 1990; Korpela 1993). Predominant RA-related underlying causes for these findings are drugs, MesGN and amyloidosis (Sellars et al. 1983; Helin et al. 1995; Nakano et al. 1998).

1.1 Proteinuria

Under physiological conditions daily urinary excretion of protein does not exceed 150 mg. However, in healthy person proteinuria may exceed the limit, as seen in fever or after exercise, idiopathic transient proteinuria, and orthostatic proteinuria (Fogazzi 2005). Daily excretion above 3.5 g is termed massive proteinuria and usually occurs when the glomeruli have been damaged seriously enough to allow plasma proteins, especially albumin, to enter the urine (Coe 1987). The combination of massive proteinuria, hypoalbuminemia, edema and hyperlipidemia is often referred to as the nephrotic syndrome (Coe 1987). Microalbuminuria is defined as an increased urinary excretion of albumin above the reference range for healthy subjects, which is undetectable by dipstick testing. Usually ranges (in two samples out of three) are 20–200 µg/min (nocturnal excretion) or 30–300 mg/day (Viberti and Wiseman 1986).
1.1.1 Prevalence and incidence of proteinuria in RA patients and in the normal population

The prevalence of proteinuria in RA patients, defined as a positive dipstick, has been reported to be 6.6-24.6 % in studies made between the 1940s and the 1960s (Fingerman and Andrus 1943; Fearnley and Lackner 1955; Sørensen 1964; Bland 1965) and 4.8-5.6% in studies from the 1980s onwards (Richards et al. 1988; Korpela 1993), while the prevalence in the normal population has been 0.7 -3.7% (Baddeley et al. 1964; Alwall and Lohi 1973; Sinniah et al. 1977; Korpela 1993; Kawamura et al. 1995). The RA patients in the studies in question were usually suffering from advanced disease and did not always receive DMARDs, or data on the medications were not provided. The reported DMARD therapy consisted mostly of gold salts, D-penicillamine (DPA), sulfasalazine and chloroquine.

If proteinuria is defined as urinary protein excretion $\geq$150-250mg/day, prevalences have ranged from 5.7-10% in advanced RA populations (Bland 1965; Korpela 1993), and an occurrence of 12.3% was found in a 5-year follow-up (Cantagrel et al. 1990). More abundant proteinuria ($\geq$500mg/d) has been found in 3.0% of RA patients and in 0.9 % of controls in a population-based cross-sectional study (Korpela 1993).

In a more recent prospective study among an early-RA population (disease duration < 1 year) patients (n=235) were assessed monthly over an average of 42 months. Proteinuria was detected in 10% and persistent proteinuria ($\geq$3 months period) in 7% of these patients (Koseki et al. 2001).

Pedersen and associates (1995) reported a prevalence of microalbuminuria (urinary albumin to creatinine ratio 3-30 mg/mmol) in 27.7% of RA patients (diabetes, hypertension and previous renal disease being excluded) and in 7.8% of controls. In a normal Australian population the prevalence of microalbuminuria (urine albumin to creatinine ratio 3.4 to 34 mg/mmol) was 6.0% (Atkins et al. 2004).
1.1.2. Associations of proteinuria in patients with RA

In RA, glomerular proteinuria has been considered a complication of advanced disease caused by the direct effects of the basic disease on the kidney, the action of nephrotoxic drugs, or both. Also concomitant diseases such as diabetes and hypertonia are to be taken into consideration in RA patients with proteinuria. The increasing duration of RA and concomitant renal functional impairment increase the probability of secondary AA-amyloidosis (Korpela 1993; Helin et al. 1995; Nakano et al. 1998). Amyloidosis has been the most common renal morphological finding in RA patients with nephrotic syndrome (Helin et al. 1995). Rheumatoid vasculitis is a rare histological diagnosis in patients with severe RA and proteinuria (Helin et al. 1995; Niederstadt et al. 1999).

An association of proteinuria and treatment with gold or DPA has been found in several studies (Pedersen et al. 1995; Niederstadt et al. 1999; Koseki et al. 2001) and the clinical finding is usually associated with the histological finding of membranotic glomerulonephritis (Hall 1982). Patients usually show normal or only mildly diminished renal function, and the duration of RA is shorter than that in patients with renal amyloidosis (Helin et al. 1995). In contrast, non-steroidal anti-inflammatory drugs (NSAIDs) rarely cause proteinuria (Pirson and van Ypersele de Strihou 1986; Pedersen et al. 1995; Koseki et al. 2001). The fact is that the renal morphologic lesion in RA patients with isolated proteinuria cannot be accurately predicted on the basis of clinical signs and symptoms, and the differential diagnosis still warrants renal biopsy to evaluate the morphological findings in patients with persistent proteinuria (Bourke et al. 1981; Helin et al. 1986; Helin et al. 1995).

Urinary albumin excretion has been correlated with high serum C reactive protein (CRP) and long duration of RA, probably reflecting high disease activity (Korpela 1993; Pedersen et al. 1995). In a study of patients with early RA, proteinuria was caused mainly by drugs (DPA, gold salts,
bucillamine, and sodium diclofenac) (Koseki et al. 2001). Risk factors for drug-induced proteinuria in the study in question were high CRP and erythrocyte sedimentation rate (ESR), and age over 50.

Proteinuria, combined proteinuria and hematuria, and also microalbuminuria (Jacobsson et al. 1993; Sihvonen et al. 2004) have been associated with an increased mortality rate in RA patients. Underlying renal amyloidosis may explain this association (Korpela 1993; Sihvonen et al. 2004).

1.2 Hematuria

Hematuria is defined as the presence of red blood cells in the urine, and bleeding in the urinary tract may arise from any site along the system. Microscopic hematuria is defined as 3 or more red cells per high-power field on microscopical examination, while gross hematuria denotes a perceptible redness of the urine (Rauta 2007). Red blood cell casts, dysmorphic erythrocytes and acanthocytes in microscopical examination of the urine indicate glomerular hematuria (Köhler et al. 1991; Roth et al. 1991). Dipstick testing for heme detects hemoglobin from 1 or 2 red blood cells per high-power field. As the presence of myoglobin or hemoglobin may result in a false-positive test result, a positive dipstick test should be confirmed by microscopic examination of urinary sediment (Cohen and Brown 2003).
1.2.1 Prevalence and incidence of hematuria in RA patients and in the normal population

Hematuria occurs frequently in patients with RA. Its incidence has been 10% to 11.7% in advanced RA populations in over 5-7 years’ follow-up (White et al. 1984; Cantagrel et al. 1990) and the cross-sectional prevalence has varied from 4.8% to 9% (Richards et al. 1988; Korpela et al. 1995). The diversity of patient populations and the different definitions of hematuria adopted in these studies have an influence on the results. The lower prevalence of hematuria (4.8%) was reported in a cross-sectional study (Richards et al. 1988) in which hematuria was defined by a single positive urine dipstick. Korpela and associates (1995) found a prevalence of 9% for isolated microscopic hematuria and no difference in prevalence was detected between the RA population and age- and sex-matched controls. Hematuria was defined in the study as a positive dipstick result in two consecutive urine samples.

White et al. (1984) reviewed retrospectively 191 RA patients treated with gold and DPA over a 7-year period. Over this period 10% showed hematuria, in most cases related to urinary tract infections. The initial positive dipstick result was confirmed by microscopic examination of urine sediment. Cantagrel et al. (1990) found in a follow-up study an occurrence of 8.0% for isolated microscopic hematuria and 11.7% for all hematuria, including samples with leucocyturia and proteinuria. Hematuria was defined as 5 red cells per high-power field or 5000 red cells/ml. The results in a prospective study by Leonard et al. (1987) clearly diverge from those in corresponding studies; a third of the RA patients treated with DPA or gold salts had repeated hematuria during the follow-up period of 20-30 weeks, and the prevalence was identical in the placebo group. Fifteen to twenty consecutive urine samples were studied and recurrent hematuria was defined as 3 or more red blood cells per high-power field found in 3 or more separate urinalyses.
In a recent prospective study of early RA (duration < one year) monthly urinalysis showed an occurrence of 18% of persistent hematuria (Koseki et al. 2001). This was defined as 5 or more red blood cells per high-power field observed during 3 months or more. Over half of the total occurrences of hematuria were intermittent.

In general population-based studies the prevalence of hematuria has ranged from 0.2 per cent to 16 per cent (Alwall and Lohi 1973; Pettersson 1982; Ritchie et al. 1986; Woolhandler et al. 1989; Hiatt and Ordonez 1994; Kawamura et al. 1995). The age and sex distribution of the screened populations, the number of tests performed per person, and also the tests used have varied substantially from study to study. Also in general population a notable proportion of the microhematuria has been found to be transient (Froom et al. 1984).

1.2.2. Associations of hematuria in patients with RA

Firstly, as in a normal population, possible nonglomerular or urological reasons for hematuria must be excluded. The most frequent renal histological finding related to recurrent isolated hematuria in RA patients has been MesGN (Hordon et al. 1984; Cantagrel et al. 1990; Korpela et al. 1995). No clinical association has been established between isolated hematuria and DMARDs (White et al. 1984; Leonard et al. 1987; Korpela et al. 1995; Koseki et al. 2001), NSAIDs (Richards et al. 1988; Korpela et al. 1995; Koseki et al. 2001) or duration of disease (Korpela et al. 1995). In a study by Korpela et al. (1995), even after thorough investigations the course of hematuria remained uncertain or unknown in half of the cases regardless of the grade of hematuria. Identical results were observed among the general population.

In one study of early RA patients (Koseki et al. 2001) hematuria was associated with older age (≥50 years) and additionally in some patients with the disease activity of RA. Likewise in an advanced
RA population hematuria was found more frequently in older (≥ 55 years) than in younger men (<55 years), while in female patients no difference was seen (Korpela 1993). Hematuria is also frequently encountered in renal amyloidosis, but is almost always combined with proteinuria (Brandt et al. 1968). No excess mortality has been detected among RA patients with isolated hematuria (Sihvonen et al. 2004).

1.3 Chronic renal failure

CRF is a clinical condition characterized by a constantly reduced GFR. CRF has been defined in the literature in a variety of ways. Earlier studies have used serum creatinine levels with variable cut-off points to determine CRF. By reason of the several limitations of serum creatinine measurement, more recent studies have switched to estimated GFR (eGFR) with a cut-off point of 60 ml/min/1.73m² (Stevens and Levey 2005). Table 1 shows the staging of chronic kidney disease (CKD).
Table 1. Staging of chronic kidney disease according to the Kidney Disease Outcomes Quality Initiative guidelines of the National Kidney Foundation of the USA (K/DOQI 2002).

<table>
<thead>
<tr>
<th>CKD stage</th>
<th>Description</th>
<th>GFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kidney damage with normal or high GFR</td>
<td>&gt; 90</td>
</tr>
<tr>
<td>2</td>
<td>Kidney damage with mildly decreased GFR</td>
<td>60-89</td>
</tr>
<tr>
<td>3</td>
<td>Moderately decreased GFR</td>
<td>30-59</td>
</tr>
<tr>
<td>4</td>
<td>Severely decreased GFR</td>
<td>15-29</td>
</tr>
<tr>
<td>5</td>
<td>Kidney failure</td>
<td>&lt;15</td>
</tr>
</tbody>
</table>

1.3.1 Prevalence and incidence of chronic renal failure in RA patients and in the normal population

In one cross-sectional population-based study (Korpela 1993) CRF was detected in 6.4% of the RA patients studied (duration of RA 15±10 years) and in 4.6% of the age- and sex-matched controls. Creatinine cut-off points of 115 µmol/l for men and 100 µmol/l for women were adopted. More advanced CRF (creatinine ≥ 150µmol/l) was found more frequently in RA patients (2.8%) than in controls (0.6%). In an unselected outpatient group of 167 advanced RA patients, serum creatinine exceeded 130 µmol/l in 3% of the patients (Richards et al. 1988). Sørensen et al. (1962) found decreased creatinine clearance in 32% of RA patients and 14% of controls. Karie et al. (2008) reported
in their prospective study of 102 RA patients (duration of RA 9.5±7.6 years) a prevalence of 18.6% for elevated serum creatinine, the upper limits for normal serum creatinine being 106 µmol/l in men and 80 µmol/l in women. An estimated GFR below 60 ml/min/1.73m² was observed in 15% and 25% of RA patients when calculated using Modification of Diet in Renal Disease (MDRD) (Levey et al. 2000) and Cockcroft-Gault (CG) (Cockcroft and Gault 1976) formulas, respectively.

In an early-RA patient group (duration of RA <1 year) a raised serum creatinine concentration (>115µmol/l in men and > 97 µmol/l µmol in women, or an increase in creatinine value by 26 µmol/l over the value recorded at entry) was detected in 6 % of patients in monthly prospective evaluation over a median 42 months (Koseki et al. 2001).

Earlier studies defining the prevalence of CRF in normal populations have used creatinine cut-off points from 124 to 150 µmol/l in women and from 133 to 150 µmol/l in men. The reported prevalence of CRF has ranged from 0.2 to 10% (Iseki et al. 1997; Jones et al. 1998; Magnason et al. 2002). More recent studies have utilized creatinine-based prediction equations (Cockcroft and Gault 1976; Levey et al. 2000) and the prevalence of eGFR < 60ml/min/1.73m² has been 4.7 -13% (Clase et al. 2002; Chadban et al. 2003; Coresh et al. 2003; Viktorsdottir et al. 2005).

1.3.2 Associations of chronic renal failure in patients with RA

Both antirheumatic drugs and complications of RA have been implicated in the etiology of renal failure. Cyclosporine and NSAIDs have been shown to cause renal functional impairment (Blackshear et al. 1985; Dijkmans et al. 1987; Weinblatt et al. 1987). Gold- or DPA-induced CRF is usually mild and temporary and is associated with membranous glomerulonephritis (Hall 1982). CRF is also attributable to concomitant hypertension and atherosclerosis (Kasiske 1987; Korpela 1993; Lamb et al. 2003).
In an early-RA patient population (Koseki et al. 2001) raised serum creatinine levels were associated predominantly with a variety of drugs, mostly other than DMARDs, e.g. diuretics, NSAIDs and angiotensin-converting enzyme (ACE) inhibitors. In more advanced disease, CRF may be a sign of renal amyloidosis and is mostly accompanied by proteinuria (Boers et al. 1987). In severe seropositive RA, renal vasculitis is a rare cause of CRF combined with proteinuria and hematuria (Scott et al. 1981).

Studies correlating the level of kidney function with the severity, activity and duration of RA show that the more severe the disease and the longer its duration, the lower the creatinine clearance will be (Sørensen 1962; Duthie et al. 1964; Sørensen 1964). Isolated CRF has been shown to be associated with ageing and hypertension in RA patients (Korpela 1993), and the level of impairment of renal function in isolated CRF was milder than that found in total CRF group also including patients with hematuria and/or proteinuria. Renal plasma flow and GFR normally decrease with ageing (Perrone et al. 1992).

Mortality has been shown to be within the expected limits in RA patients with isolated CRF, whereas CRF associated with other renal findings predicts increased mortality (Sihvonen et al. 2004).

2. RA-associated renal diseases

In renal biopsy studies performed among RA patients with clinical renal disease the most frequent morphological findings have been MesGN, membranotic glomerulonephritis and amyloidosis (Ørjavik et al. 1981; Sellars et al. 1983; Helin et al. 1995; Nakano et al. 1998).
2.1 Renal AA-amyloidosis

Secondary AA-amyloidosis is a well-known complication of chronic inflammatory diseases such as RA (Husby 1985; Falk et al. 1997). Amyloidoses form a group of diseases characterized by extracellular deposition of proteins in characteristic insoluble amyloid fibrils, leading to organ dysfunction, organ failure and eventually to death (Falk et al. 1997). AA-amyloid fibrils are derived from the circulating acute-phase reactant serum amyloid A protein (SAA). A persistently high serum concentration of SAA is a prerequisite for the development of AA amyloidosis (Gillmore et al. 2001), but other poorly understood genetic or molecular mechanisms also have an influence on the deposition of amyloid (Hazenberg and van Rijswijk 1994; Lachmann et al. 2007). The period of latency between the onset of inflammation and clinical presentation with AA amyloidosis is reported to be 16-18 years (Hazenberg and van Rijswijk 1994; Kobayashi et al. 1996; Uda et al. 2006; Lachmann et al. 2007).

Renal involvement dominates the clinical course in patients with AA-amyloidosis (Gertz and Kyle 1991; Joss et al. 2000; Lachmann et al. 2007). Renal amyloidosis generally presents as progressive proteinuria leading to nephrotic syndrome and renal impairment (Boers et al. 1987). The appearance of hematuria is also possible, but usually combined with proteinuria (Brandt et al. 1968; Korpela 1993; Helin et al. 1995; Nakano et al. 1998).
The diagnosis of amyloidosis is based on histologic analysis. Samples are stained with Congo red and analyzed under polarized light. Apple-green birefringence is considered evidence of the presence of amyloid (Bennhold 1922; Divry and Florkin 1927). Under the electron microscope, amyloid deposits appear as rigid, non-branching and randomly arranged fibrils 8-10 nm in diameter (Merlini and Bellotti 2003). Biopsy of an involved organ is the diagnostic golden standard. In the kidney, the mesangium is the first part of the glomerus in which amyloid can be demonstrated, followed in later stages by deposits in the walls of capillaries and tubules and also in the walls of interstitial blood vessels (Van der Hem and van Rijswijk 1992) (Figure 1). Glomerular involvement appears to be associated with severe proteinuria or renal failure (Looi 1989) and predominantly vascular amyloidosis with minimal or no proteinuria and severe loss of renal function (Falck et al. 1983). On the other hand, good preservation of renal function associated with the predominantly vascular amyloidosis has also been recorded in cases where glomerular amyloid deposition was totally absent (Uda et al. 2006).

Clinical suspicion being high, the simplest and safest way to confirm the diagnosis is to obtain a fine-needle aspiration biopsy of subcutaneous abdominal fat (Westermark and Stenkvist 1973; Libbey et al. 1983). Amyloid deposition in abdominal fat tissue is seen exclusively in the setting of systemic amyloidosis (Libbey et al. 1983). The specificity of this test approaches 100%, whereas its sensitivity varies greatly, from 52% to 88% (Libbey et al. 1983; Duston et al. 1989; Masouye 1997; Guy and Jones 2001).
Figure 1. Glomerulus stained with Congo red and viewed under polarized light. The characteristic apple-green birefringence for amyloid is seen.

Figure 2. A renal biopsy sample reveals slight mesangial hypercellularity in a glomerulus of RA patient with mesangial glomerulonephritis (PAS-hematoxylin staining)

The pictures are provided by Dos. Heikki Helin, Helsinki University Hospital, Finland
Early and aggressive treatment of the underlying inflammation is fundamental in the prevention of AA amyloidosis and also in retarding its progression in RA. Treatment holding the circulating SAA concentration at low values can even lead to regression of AA amyloid deposits and also prolongation of survival (Lachmann et al. 2007). A favorable effect of the alkylating agents (Ahlmen et al. 1987; Berglund et al. 1993; Chevrel et al. 2001) and also modern therapies such as TNF-α inhibitors on the prognosis of AA-amyloidosis in patients with inflammatory arthritis has been shown (Elkayam et al. 2002; Gottenberg et al. 2003).

Since the decrease in the prevalence of chronic infectious diseases, chronic rheumatic diseases including RA, have become the most common conditions inducing systemic AA-amyloidosis in the more developed countries (Browning et al. 1985; Hazenberg and van Rijswijk 1994; Joss et al. 2000; Bergesio et al. 2007; Lachmann et al. 2007). Nonetheless, during the last two decades, the clinical impression has emerged that the incidence of AA-amyloidosis secondary to RA is declining. In the Rheumatism Foundation Hospital in Heinola, Finland, the proportion of amyloidosis findings among all biopsies obtained decreased from 10.2% in 1987-1989 to 5.1% in 1997 (Laiho et al. 1999). In other studies the prevalence of amyloidosis in rheumatic diseases has been higher, 5-20% (Dhillon et al. 1989; Tiitinen et al. 1993; Kobayashi et al. 1996; Myllykangas-Luosujärvi et al. 1999; Gomez-Casanovas et al. 2001; Kuroda et al. 2002). According to the Finnish Registry for Kidney Diseases (2007) the number of amyloidosis patients entering renal replacement treatment (i.e. dialysis and kidney transplantation) has clearly decreased since 2000.

Renal amyloidosis is associated with over 2-fold mortality compared to cases yielding normal renal findings (Sihvonen et al. 2004). Altogether, the lifespan of RA patients with amyloidosis has been shown to be shortened by 7.7 years (Myllykangas-Luosujärvi et al. 1999). Nonetheless, the survival of patients with AA-amyloidosis has improved in the last decades; Gerz et al. (1991) reported a median
survival of 24 months, Joss et al. (2000) 53 months, Lachmann et al. (2007) 133 months and Bergesio et al. (2008) 79 months.

2.2 Mesangial glomerulonephritis (MesGN)

The presence of mild endocapillary proliferative glomerulonephritis in RA, formerly designated rheumatoid glomerulitis, was described in autopsy studies in the 1940s and 1950s (Baggenstoss and Rosenberg 1943; Fingerman and Andrus 1943; Cruickshank and Sinclair 1956). In renal biopsy materials, MesGN has been a frequent finding in RA patients with hematuria and/or proteinuria (Pasternack et al. 1967; Ørjavik et al. 1981; Sellars et al. 1983; Hordon et al. 1984; Helin et al. 1986; Pollet et al. 1989) but only rarely associated with nephrotic syndrome (Helin et al. 1986; Helin et al. 1995). Nonetheless, the finding of MesGN in association with isolated hematuria is by no means specific to RA, as it also frequently causes hematuria in nonrheumatic patients (Sinniah et al. 1977; Pardo et al. 1979; Pettersson 1982).

Histological diagnosis is based on renal biopsy specimen examination by light-, electron- and immunofluorescence microscopy, and abnormal findings in at least two of the three methods are required (Helin et al. 1986). The most frequent light-microscope lesion is mild mesangial hypercellularity with or without a slight increase in the mesangial matrix (Figure 2). Immunofluorescence study most usually shows mesangial deposits of immunoglobulin (mostly IgM and IgA, rarely IgG) with or without associated complement C3 (rarely C1q). In electron microscopy examination, electron-dense deposits are mostly located in the mesangial area between the basement membrane and the mesangial cell cytoplasm. Especially when mild glomerular lesions are analyzed, immunofluorescence and electron microscopy significantly increase the accuracy and objectivity of interpretation (Helin et al. 1986).
Isolated hematuria in RA patients with MesGN seems to be unrelated to DMARD treatment (Hordon et al. 1984; Korpela et al. 1991; Nakano et al. 1998), but DMARDs (e.g. gold salts, DPA) may contribute to proteinuria in these subjects (Korpela et al. 1991). There is no evidence regarding NSAID-induced MesGN (Korpela et al. 1991). High RF titers have correlated with the presence of MesGN (Sellars et al. 1983; Helin et al. 1986; Korpela et al. 1997). A significant correlation with the intensity of mesangial IgM deposits and the levels of serum IgM-RF has been found, and also a correlation between the intensity of mesangial IgA and the duration and severity of RA (Korpela et al. 1997). RFs may be involved in the pathogenesis of renal injury in patients with MesGN. Thus, MesGN is considered to be related to the basic rheumatoid disease and regarded as an extra-articular manifestation of RA (Korpela et al. 1997). A hypothesis has also been introduced suggesting that a functional response by the glomerular mesangium to remove circulating IgM-RF-IgG immunocomplexes could lead to MesGN (Pollet et al. 1989).

Previous studies (median duration of follow-up 3.5-7.7 years) have demonstrated that in RA patients with MesGN presenting with microscopic hematuria, renal function does not deteriorate during the follow-up, although in most cases hematuria persists constantly (Kelly et al. 1988), whereas MesGN presenting with proteinuria is associated with poorer prognosis (Korpela et al. 1991). Mortality has been shown to be within the expected limits in RA patients with MesGN (Sihvonen et al. 2004).
2.3 Other diseases

Systemic rheumatoid vasculitis is considered a rare serious complication of long-standing seropositive RA associated with a wide range of other extra-articular features and high levels of IgG- and IgM – RFs. Renal vasculitis is reported to be present in approximately one quarter of patients with systemic rheumatoid vasculitis (Scott et al. 1981). In one autopsy study, large-vessel renal vasculitis and extracapillary proliferative glomerulonephritis were described as histological findings (Boers et al. 1987), but there are also reports of a necrotizing and crescentic glomerulonephritis with the occasional presence of perinuclear antineutrophil cytoplasmic antibodies (p-ANCA) (Breedveld et al. 1985; Kuznetsky et al. 1986; Harper et al. 1997).

Although renal tubular dysfunction is common in patients with RA, it is not regarded as a specific complication of the disease (Boers et al. 1987). It has been associated with number of factors such as increased age, urinary tract infection, severe concomitant illness and use of NSAIDs or diuretics (Hordon et al. 1991), and also with disease activity in RA (Dieppe et al. 1976). The condition is usually manifested with proteinuria and/or increased excretion of urinary tubular enzymes (Morgan 1982).
3. Renal diseases related to DMARDs and NSAIDs

3.1 Gold salts and D-penicillamine

Gold salts and DPA were DMARDs widely used in the past, especially up to the 1980s. The mechanism of action of these compounds in the treatment of RA is not completely understood. Gold salts appear to inhibit monocyte proliferation, diminish monocyte phagocytic function, and inhibit the production of immunoglobulin M and rheumatoid factor. The use of DPA is associated with decreased B-cell function and reduced T-cell proliferation (Gardner and Furst 1995).

Gold and DPA treatments are frequently complicated by renal side-effects, presenting as proteinuria, this often leading to discontinuation of the treatment (Hall 1982). During gold therapy proteinuria occurs in 2-10% of patients and nephrotic syndrome in up to one third of them (Hall 1982). Gold-induced proteinuria has been reported with both parenteral and oral gold treatment (Watanabe et al. 1976; Revach et al. 1979). During DPA treatment of RA patients, proteinuria has been observed in up to 30%, while proteinuria has been sufficiently severe to cause nephrotic syndrome in less than 20% of affected patients (Hill 1977). Proteinuria resolves spontaneously within 2-3 years of discontinuing the gold or DPA treatment and severe or progressive deterioration of renal function has not been observed. The median duration of proteinuria has been less than one year (Hall et al. 1987; Hall et al. 1988).

Renal biopsy specimens taken shortly after the onset of proteinuria during DPA or gold therapy have in most cases shown membranous glomerulonephritis (Silverberg et al. 1970; Tönroth and Skrifvars 1974; Bacon et al. 1976; Hall et al. 1987; Hall et al. 1988), although the condition can also occur without prior DMARD therapy in RA patients (Higuchi et al. 1987; Nakano et al. 1998). In silver
methenamine-stained sections, glomerular capillary basement membranes show silver-negative vacuoles, spikes and possibly early thickening. Under electron microscopy, subepithelial electrondense deposits are seen. Immunofluorescence microscopy usually shows small granular deposits of IgG. The pathogenesis of gold- and DPA-induced membranous glomerulonephritis is poorly understood, but release of an autoantigen from the renal tubular epithelium has been discussed (Skrifvars 1979). In addition to membranous glomerulonephritis, gold and DPA therapy have also been accompanied by occasional immune complex mesangial glomerulonephritis, minimal change nephritis (Hall et al. 1987; Hall et al. 1988) and p-ANCA-associated crescentic glomerulonephritis (due to DPA treatment) (McCormick et al. 1977; Almirall et al. 1993).

3.2 Cyclosporine

Cyclosporine is an immunomodulative agent which inactivates calcineurin, leading to blockage of the gene transcription for specific cytokines, particularly interleukin-2 and γ-interferon. Thus, the secretion of cytokines which normally occurs during T-cell activation is inhibited (Wiederrecht et al. 1993). The utility of cyclosporine as a second-line DMARD for RA patients has been shown, but the renal toxicity of the drug has limited its use. Renal toxicity has been manifested primarily as a significant increase in plasma creatinine levels and a subsequent decline in creatinine clearance, which has returned to normal in most cases after discontinuation of cyclosporine (Dijkmans et al. 1987; Weinblatt et al. 1987). The reduction in renal function is considered to be a correlative of cyclosporine-induced renal afferent arteriolar vasoconstriction with a resultant reduction in renal blood flow (Mason 1992).

Renal histological abnormalities associated with cyclosporine include focal interstitial fibrosis with tubular atrophy, arteriolar alterations, or both (Feutren 1993). The major risk factors identified are increasing age, the use of cyclosporine dosages > 5mg/kg/day, an increase in creatinine levels 50% of
values before administration of cyclosporine, and the occurrence of hypertension during treatment (Feutren and Mihatsch 1992). Rodriguez et al. (1996) reported in their renal biopsy study an occurrence of 10% of these representative histological findings in RA patients treated with cyclosporine.

3.3 Other disease-modifying antirheumatic drugs (DMARDs)

Methotrexate is a dihydrofolate reductase inhibitor widely used in the treatment of RA. Subtle changes in renal function associated with this treatment have been reported in RA patients. The reduction in creatinine clearance or $^{51}$Cr-EDTA clearance has been around 10% (Seideman et al. 1993; Kremer et al. 1995). As 90% of a dose of methotrexate is eliminated by the kidneys, even a small compromise in renal function could increase serum levels of methotrexate and thus increase the potential for methotrexate toxicity, particularly in the elderly (Wolfe and Cathey 1991; Kremer et al. 1995).

The antimalarial drugs, especially hydroxychloroquine, are frequently used in DMARD combinations in the treatment of RA. The antimalarials are thought to interfere with antigen recognition by T-helper cells. Their safety profile is considered favorable, but in one retrospective study a significant decrease in mean creatinine clearance from 99ml/min to 92 ml/min was reported (Landewe et al. 1995). Because up to 40% of a dose of hydroxychloroquine is excreted unchanged in the urine, patients with abnormal renal function may have an increased risk of adverse events, especially retinal damage (Mackenzie 1983).

Sulphasalazine inhibits inflammation by inhibition of cyclo-oxygenase (COX) and lipoxygenase pathways, by inhibiting inflammatory cytokines, and by interfering with cellular activation (Gardner and Furst 1995). The renal toxicity of sulfasalazine has not been a concern in the treatment of RA patients (Jones et al. 1991), but nephrotic syndrome, minimal change nephropathy and
interstitial nephritis have been reported to be associated with its use in patients with ulcerative colitis (Barbour and Williams 1990; Dwarakanath et al. 1992).

The pro-inflammatory cytokine TNF-α has a key role in the pathogenesis and progression of RA (Arend and Dayer 1995), and it is nowadays the main target for biological DMARDs. There are currently three most widely used anti-TNF-α agents: adalimumab, etanercept and infliximab. These agents are generally well tolerated and serious side-effects are rare (Khanna et al. 2004). Renal involvement is unusual, but a variety of renal pathologic findings have been described related to anti-TNF-α therapies, including proliferative lupus nephritis, pauci-immune necrotizing and crescentic glomerulonephritis, membranous glomerulonephritis with renal vasculitis and extracapillary glomerulonephritis (Stokes et al. 2005; Saint Marcoux and De Bandt 2006; Simms et al. 2008). The clinical manifestations of renal disease have been new-onset proteinuria (also nephrotic-range), hematuria, and/or renal dysfunction.

An etiologic role for the anti-TNF-α agents in the initiation of glomerulonephritis has been discussed. Nonetheless, the temporal relation of new-onset glomerular disease to anti-TNF-α therapy in patients with long-standing RA and the improvement in clinical symptoms seen after drug withdrawal support such an etiologic role. In addition, the clinical symptoms are often accompanied by formation of new autoantibodies such as antinuclear, anti-double stranded DNA, ANCA, and antiphospholipid or anticardiolipin antibodies (Stokes et al. 2005; Saint Marcoux and De Bandt 2006). However, in RA patients with renal amyloidosis, a favorable influence of the anti-TNF-α agents on proteinuria and renal function has been reported (Elkayam et al. 2002; Gottenberg et al. 2003).

Most RA patients with active disease are treated with a combination of several classes of drugs. Polypharmacy is associated with the risk of additive and synergistic nephrotoxicity (Schiff and Whelton 2000), e.g. patients taking NSAIDs and cyclosporine together may experience severe nephrotoxicity (Landewe et al. 1994). Also concomitant use of multiple DMARDs involves a risk of
additive or synergistic toxicity, although no overt serious renal toxicity has been reported (Schiff and Whelton 2000).

3.4 Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs suppress the production of prostaglandins from arachidonic acid usually by blocking both COX-1 and COX-2 enzymes. These enzymes have a major significance in the activation of compensatory renal hemodynamic mechanisms in the clinical setting of reduced renal perfusion. Patients at risk of acute renal functional deterioration while using NSAIDs include those with severe heart disease (Walshe and Venuto 1979), severe hepatic disease (Oates et al. 1988), nephrotic syndrome (Arisz et al. 1976), chronic renal disease, dehydration (Blackshear et al. 1985) and advanced age (Whelton and Hamilton 1991). Prompt discontinuation of NSAID therapy usually restores renal function (Blackshear et al. 1985). Also COX-2-selective NSAIDs have been shown to induce a vasomotor type of renal function deterioration (Perazella and Eras 2000). Furthermore, sodium and fluid retention are common NSAID-related renal complications, whereas hyperkalemia can occur in specific at-risk patients (Whelton and Hamilton 1991).

A sudden onset of proteinuria combined with interstitial nephritis and renal insufficiency is an uncommon but distinct NSAID-related renal syndrome (Clive and Stoff 1984). Such patients typically have glomerular lesions consistent with minimal change disease and an associated acute allergic interstitial nephritis. The proteinuria is usually nephrotic-range (Bender et al. 1984), and the condition generally develops in patients with pre-existing normal renal function (Clive and Stoff 1984). Almost all nonselective NSAIDs have been reported to cause nephrotic syndrome, but also reports on COX-2 selective NSAIDs have been published (Alper et al. 2002). After discontinuation of the NSAID, most
patients have a spontaneous remission within 1 month, although proteinuria may last for up to 1 year (Clive and Stoff 1984).

Renal papillary necrosis is an uncommon, but permanent form of renal parenchymal damage. Acute renal papillary necrosis is a consequence of short-term ingestion of excessive doses of an NSAID, invariably during a time of severe dehydration (Whelton 1999). The underlying pathophysiologic process appears to be ischemic necrosis (Atta and Whelton 1997). Chronic renal papillary necrosis is a part of the entity of analgesic abuse nephropathy, which is caused by overuse of mixtures of analgesics consumed for 5-20 years (Whelton 1999). This type of injury is mostly to be attributed to phenacetin, but other analgesic mixtures not containing phenacetin can also produce analgesic abuse nephropathy (Elseviers and De Broe 1996). In addition to papillary necrosis, the nephropathy is characterized by chronic interstitial nephritis. Symptoms begin with hematuria, sterile pyuria, and possibly with renal colic subsequently followed by hypertension and moderate renal failure (Nanra 1983).

4. Measurement of renal function in RA patients

4.1 Chronic kidney disease and glomerular filtration rate

GFR is the product of the filtration rate in single nephrons and the number of nephrons in both kidneys. It is widely accepted as the best overall measure of kidney function (Stevens and Levey 2005). Chronic kidney disease (CKD) is defined and categorized according to the Kidney Disease Outcomes Quality
Initiative guidelines of the National Kidney Foundation of the USA (K/DOQI 2002) (Table 1, page 22).

The diagnosis of CKD presumes kidney damage for 3 months or more, as defined by structural or functional abnormalities in the organ (e.g. proteinuria or abnormalities in imaging studies or on kidney biopsy), with or without decreased GFR, or GFR below 60 ml/min/1.73m$^2$ for 3 months or more with or without kidney damage.

The normal values of GFR are approximately 130 ml/min/1.73m$^2$ in men and 120 ml/min/1.73m$^2$ in women under the age of 30 years (Wesson 1969). These figures are related to age, sex and body size and are also affected by normal physiological stages such as pregnancy (Stevens and Levey 2005). GFR declines with age, the average decline being 10 ml/min/1.73m$^2$ per decade after the age of 30 years (Davies and Shock 1950), whereas the definition of CKD does not vary with age. An estimated GFR below 60 ml/min/1.73m$^2$ is considered an independent predictor of cardiovascular disease and death (Manjunath et al. 2003; Sarnak et al. 2003) and also necessitates adjustment of medical treatment.

4.2 The significance of estimating glomerular filtration rate in RA patients

It is important for a clinician to be aware of RA patients’ precise estimate of GFR. A reduced GFR below 60 ml/min/1.73m$^2$ has an essential influence on selection and dosage of medications to avoid adverse events and further damage to kidneys. The dosage of DMARDs excreted by the kidneys (e.g. methotrexate) should be adjusted and nephrotoxic drugs (cyclosporine, NSAIDs) avoided. Inadequate attention is paid to this issue. In the MATRIX (methotrexate and renal insufficiency study), which involved 129 unselected RA patients (RA duration 9.5 ±7.6 years), most subjects with GFR <60 ml/min/1.73m$^2$ received at least one drug which required dose adjustment. Additionally, 70% of these patients received at least one drug which was potentially nephrotoxic. Half of the patients receiving
methotrexate treatment did not have appropriate dosage adjustment according to their stage of CKD (Karie et al. 2008).

4.3 Measurement of exogenous substances

Inulin, a 5200-d uncharged polymer of fructose, is the gold standard for filtration marker. It is freely filtered in the glomerulus, is inert, does not alter kidney function, and is neither metabolized nor reabsorbed. However, measurement of GFR by inulin is somewhat laborious and inconvenient, requiring continuous intravenous infusion of inulin, urine collection by bladder catheterization, and measurement under standard conditions. Thus, alternative less complex methods, e.g. $^{51}$Cr-EDTA, have been developed and validated (Levey 1990). These measurements are nonetheless still too cumbersome and expensive to be used in clinical practice and endogenous filtration markers are needed.

4.4 Plasma creatinine and creatinine clearance

The plasma concentration of creatinine is the most widely used endogenous marker of renal function in clinical medicine. The concentration is easy to measure, and the means inexpensive and widely available (Stevens and Levey 2005). There is a reciprocal relationship between the steady-state plasma level of creatinine and GFR (Kassirer 1971), but this is subject to interference from several factors, which renders creatinine less than ideal as a filtration marker (Perrone et al. 1992).

Creatinine is an end product of muscle catabolism and its generation is mostly proportional to the total muscle mass, which is in turn related to age, sex, race and conditions causing muscle wasting.
Also alterations in the dietary intake of meat, which is the most important source of external creatinine, have an influence on the creatinine pool (Perrone et al. 1992). Creatinine is freely filtered in the glomerulus, and actively secreted in the proximal tubule, especially in the presence of renal insufficiency. Also tubular reabsorption is possible (Perrone et al. 1992). Significant extrarenal elimination of creatinine by bacterial degradation in the gastrointestinal tract may occur in patients with severe CKD (Levey et al. 1988). These issues make measurement of serum creatinine a rather insensitive marker of reduced GFR, and it does not usually exceed the normal range until as much as 50 per cent of total GFR is lost (Brenner et. al. 1987).

The standard method of measurement of serum creatinine is the alkaline picrate (Jaffe) reaction (Jaffe 1886). The assay is prone to interference from substances in the serum, particularly proteins (noncreatinine cromogens), leading to possible overestimation of serum creatinine by as much as 15-25%. Enzymatic creatinine methods have been adopted for clinical laboratory use to reduce the interferences related to the Jaffe methods (Myers et al. 2006). Regardless of the level of creatinine, enzymatic creatinine methods show approximately 10 -20 µmol/l lower values than the Jaffe (Harmoinen et al. 1991; Myers et al. 2006).

Use of the serum concentration of creatinine as an index for GFR is especially problematic in patients with RA. Due to inactivity and inflammation, a longer duration of RA may lead to a decrease in muscle mass without a concomitant reduction in total body weight (Herbison et al. 1987; Miro et al. 1996), this leading to overestimation of renal function. The serum creatinine level itself is not a reliable marker of renal function in patients with RA (Nived et al. 1983; Boers et al. 1988) and the mean urinary creatinine secretion has been shown to be lower than in a control population (Boers et al. 1988).

After urine collection for 24 hours, creatinine clearance (ml/s) can be calculated from urine volume, collection time and serum creatinine and urine creatinine concentrations. Endogenous creatinine clearance has been considered to be a more sensitive marker of reduced renal function than
measurement of serum creatinine (Levey et al. 1988). However, measurement of creatinine clearance necessitates 24-hour collection of urine, making it laborious and also prone to collecting inaccuracies. Creatinine clearance exceeds the GFR by 10 to 20 ml/min/1.73m$^2$ by reason of tubular secretion. The amount of overestimation varies among and within individual persons (Levey et al. 1988). Despite the several interfering factors reports of the good sensitivity of endogenous creatinine clearance and correlation with measured GFR have also been published (Table 2, page 46) (Stevens et al. 2006).

4.5 Creatinine-based prediction equations

More than 25 formulas and nomograms have been developed to bypass the limitations inherent in the use of creatinine alone as a filtration marker (K/DOQI 2002). Estimating equations include variables such as age, race and body size, in addition to serum creatinine, as surrogates for muscle mass. An estimation equation is derived using regression techniques to model the observed relation between the serum level of the marker and the measured GFR in a study population. Even if an equation developed in one population is appropriate for use in that population this is not necessarily the case in a different patient cohort (Stevens et al. 2006). In patient populations not included in those in which the estimations were developed (e.g. extremes of age and body size, diseases of skeletal muscle, tetra- or paraplegia, rapidly changing kidney function, pregnancy) the most appropriate estimate of kidney function may be 24-hour urine collection for the measurement of creatinine clearance (Stevens and Levey 2005).

Perhaps the simplest and most widely used approach is the formula of Cockcroft-Gault (CG) (Cockcroft and Gault 1976):
eGFR/C\(G\) = \(\frac{(140-\text{age}) \times \text{body weight (kg)}}{\text{plasma creatinine (\text{\textmu mol/l})} \times a}\),

where \(a = 0.8\) if male, and \(0.95\) if female.

The CG formula was originally developed to predict creatinine clearance, but is nowadays widely used as an estimate of GFR. The equation was derived from investigation of 249 hospitalized patients (239 men and 10 women) without evidence of renal disease. Serum creatinine was originally determined by the kinetic alkaline picrate (Jaffe) method. The formula systematically overestimates GFR in consequence of the tubular secretion of creatinine. Additionally, the use of enzymatically determined creatinine in the formula leads to higher estimated GFR levels than does the use of the original Jaffe’s creatinine method.

In RA patients the predicted creatinine clearance by CG has been found to exceed the measured creatinine clearance by 20 ml/min, independent of the measured value (Boers et al. 1988), while Anders et al. (2000) found no overestimation. However, the overall performance of the CG formula in predicting creatinine clearance or GFR has been fairly satisfactory in RA patients (Boers et al. 1988; Boers et al. 1994; Laiho et al. 2001).

The MDRD (Modification of Diet in Renal Disease) formula (Levey et al. 2000) is a more recent equation for the estimation of GFR adjusted for \(1.73m^2\), which has been developed using data from a patient population with CKD (mean GFR 40 ml/min/1.73m\(^2\)):

\[
eGFR/MDRD = 186 \times (\text{plasma creatinine (\text{\textmu mol/l})/88.4})^{-1.154} \times \text{age}^{-0.203} \times (0.742 \text{ if female}).
\]

The equation is in abbreviated form, which excludes serum urea and albumin.

The MDRD and CG equations have been evaluated in numerous different patient populations (Poggio et al. 2005). In some studies, MDRD has been reported to be more accurate than CG (Rule et al. 2004; Froissart et al. 2005; Poggio et al. 2005; Rigalleau et al. 2005), whereas other studies have found their performance equal or the CG formula to be better (Vervoort et al. 2002; Ibrahim et al. 2005;
Verhave et al. 2005). The performance of these equations greatly depends on the population evaluated. Using CG formula in obese individuals can lead to gross overestimation of GFR, since weight is a numerator in the formula (Lamb et al. 2005). Both formulas perform well in populations with CKD but are less accurate in populations without CKD (Coresh and Stevens 2006). Thus, it has even been recommended not to report numerical values for an estimated GFR >60 ml/min/1.73m$^2$ (Myers et al. 2006).

There are more plentiful data on the usefulness of the CG formula than MDRD in RA populations (Table 2). Anders et al. (2002) found CG to be superior to MDRD in predicting creatinine clearance in patients with RA (Table 2), but the original MDRD formula containing urea and albumin was adopted instead of the abbreviated version. Knowledge of the usefulness of the MDRD formula in the estimation of GFR in RA patients is thus scant and it has not been compared to direct measurement of GFR in RA patients.

4.6 Plasma cystatin C

Cystatin C is a novel endogenous filtration marker which has even been considered as a potential replacement for serum creatinine (Newman et al. 1994; Grubb 2000; Dharnidharka et al. 2002). Cystatin C is a 13 kDa protein, a cysteine proteinase inhibitor, which is produced at a relatively constant rate in all nucleated cells (Abrahamson et al. 1987). Its production has not been reported to be affected by sex, muscle mass or inflammation (Abrahamson et al. 1990; Grubb 1992; Finney et al. 2000), although opposite results have also been recorded (Knight et al. 2004; Macdonald et al. 2006). Cystatin C is freely filtrated (Grubb 1992), completely reabsorbed and catabolized by the proximal tubule cells and it does not involve renal tubular secretion (Tenstad et al. 1996). Automated immunoassays have been developed based on the particle-enhanced turbidimetric immunoassay
PETIA) (Kyhse-Andersen et al. 1994) or the particle-enhanced nephelometric immunoassay (PENIA) (Finney et al. 2000).

Several studies have compared serum levels of cystatin C and creatinine as filtration markers in different kinds of populations with varying renal function, and most have found serum cystatin C to be a better estimate of GFR than serum creatinine (Dhamidharka et al. 2002; Laterza et al. 2002). In general, it seems that cystatin C may have an advantage in detecting mildly decreased GFR, whereas serum creatinine may be better at lower levels of GFR (Stevens and Levey 2005).

Despite some possible advantages of cystatin C, several studies have suggested that a number of factors other than GFR might influence its serum concentration. In a study by Knight and associates (2004) a significant association of cystatin C concentration with age, male sex, increased weight and height, smoking and inflammation was recorded, although in that study GFR was not measured directly in that study. Cystatin C has also been claimed to be dependent on body composition, especially total lean mass (Macdonald et al. 2006). The extrarenal excretion of cystatin C may increase at reduced GFR (Sjöström et al. 2005; Madero et al. 2006). Glucocorticoid therapy leads to a transitory and dose-dependent increase in cystatin C levels (Cimerman et al. 2000; Risch et al. 2001; Wasen et al. 2003; Bokenkamp et al. 2007). Serum cystatin C has been shown to decrease in hypothyroidism and increase in hyperthyroidism (Manetti et al. 2005). RF possibly interferes with the cystatin C assay by a non-specific agglutination of the Fc region of the immunoglobulin G molecules, increasing the apparent concentration of cystatin C (Lamb and Stowe 2003). However, Kyhse-Andersen et al. (1994) found no correlation between the concentrations of RF and of cystatin C.

There has been increasing interest in the development of equations based on cystatin C to predict GFR in ml/min (Hoek et al. 2003; Larsson et al. 2004; Grubb et al. 2005; Sjöström et al. 2005; Rule et al. 2006), but the clinical use of cystatin C-based estimation equations is not at present established practice (Madero et al. 2006).
Knowledge of the usefulness of cystatin C as a marker of GFR in patients with RA is scant (Table 2). The sole comparison between creatinine and cystatin C measurements was made using creatinine clearance as a reference method. Cystatin C showed a better correlation with creatinine clearance than did serum creatinine (Mangge et al. 2000). However, possible interference from RF, glucocorticoid therapy and body composition remains a source of concern in the estimation of renal function by cystatin C in RA patients.

4.7 Plasma urea

Urea is an end product of protein catabolism and is freely filtered by the glomerulus and passively absorbed in proximal and distal nephrons (Forster 1970), and excreted in high concentration in the urine. Extracellular fluid depletion causes increased urea reabsorption, leading to a greater decrease in urea clearance than in concomitant GFR, and leading also to higher serum concentrations of urea (Stevens and Levey 2005). Urea is synthetized by the liver and this process is enhanced by e.g. a protein rich diet, infections, congestive heart failure and use of glucocorticoids or diuretics. Any catabolic situation (e.g. infection or hypothyreosis) may cause increase in urea production, whereas severe malnutrition and liver disease reduce the production of urea. By reason of several interfering factors neither the serum urea level nor its clearance is now used as an index of kidney function (Stevens and Levey 2005) and the situation is similar among patients with RA. Likewise no specific data on the reliability of serum urea measurement as an index for GFR in RA patients are available.
Table 2. Correlation coefficients of different GFR estimates in RA patients and controls compared to direct measure of GFR or creatinine clearance

<table>
<thead>
<tr>
<th>Compared measures</th>
<th>N</th>
<th>r in RA patients (test)</th>
<th>r in controls</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crea vs Creacl</td>
<td>54</td>
<td>-0.72 (Pearson’s)</td>
<td></td>
<td>Laiho et al. 2001</td>
</tr>
<tr>
<td>Crea vs CG</td>
<td>54</td>
<td>-0.71 (Pearson’s)</td>
<td></td>
<td>Laiho et al. 2001</td>
</tr>
<tr>
<td>Crea vs Creacl</td>
<td>56</td>
<td>-0.31 (Kendall’s tau)</td>
<td></td>
<td>Mangge et al. 2000</td>
</tr>
<tr>
<td>CreaCl vs $^{51}$Cr-EDTA cl</td>
<td>167</td>
<td>0.88 (Spearman rank)</td>
<td></td>
<td>Richards et al. 1988</td>
</tr>
<tr>
<td>CreaCl vs $^{125}$I-thalamate</td>
<td>35</td>
<td>0.72 (Kendall’s tau)</td>
<td></td>
<td>Boers et al. 1990</td>
</tr>
<tr>
<td>CreaCl vs $^{125}$I-thalamate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>and $^{131}$I-hippurate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG vs Creacl</td>
<td>27</td>
<td>0.91 (linear regression)</td>
<td>0.92</td>
<td>Boers et al. 1988</td>
</tr>
<tr>
<td>CG vs Creacl</td>
<td>38</td>
<td>0.69 (linear regression)</td>
<td>0.89</td>
<td>Anders et al. 2000</td>
</tr>
<tr>
<td>CG vs Creacl</td>
<td>54</td>
<td>0.80 (Pearson’s)</td>
<td></td>
<td>Laiho et al. 2001</td>
</tr>
<tr>
<td>CG vs Creacl</td>
<td>33</td>
<td>0.69 (linear regression)</td>
<td>0.82</td>
<td>Anders et al. 2002</td>
</tr>
<tr>
<td>CG vs $^{125}$I-thalamate and $^{131}$I-hippurate or $^{51}$Cr-EDTA cl</td>
<td>122</td>
<td>0.82 (linear regression)</td>
<td></td>
<td>Boers et al. 1994</td>
</tr>
<tr>
<td>MDRD vs Creacl</td>
<td>33</td>
<td>0.41 (linear regression)</td>
<td>0.83</td>
<td>Anders et al. 2002</td>
</tr>
<tr>
<td>Cyst C vs Creacl</td>
<td>56</td>
<td>-0.49 (Kendall’s tau)</td>
<td></td>
<td>Mangge et al. 2000</td>
</tr>
</tbody>
</table>

CG= Cockcroft Gault formula  
Crea = creatinine  
Creacl= creatinine clearance  
CystC= cystatin C  
MDRD= Modification of Diet in Renal Diseases  
N= number of patients  
r= correlation coefficient
AIMS OF THE STUDY

The aims of this study were to establish:

1. the long-term outcome of abnormal renal findings (proteinuria, hematuria and chronic renal failure) diagnosed in the cross-sectional population-based study in 1988, the outcome of clinical renal disease and the incidence of new abnormal renal findings in the median 13 years’ follow-up period in patients with advanced RA

2. the long-term prognosis of RA-related renal diseases, especially MesGN and AA-amyloidosis, in the 13 to 15 years’ follow-up time

3. the diagnostic accuracy of conventional creatinine-based methods (the concentration of plasma creatinine, endogenous creatinine clearance, creatinine-based prediction equations) and the concentration of plasma cystatin C in estimating renal function in RA patients

4. the cumulative incidence of abnormal clinical renal findings in patients with early RA and the renal safety of initial intensive treatment with a combination of DMARDs in early RA (11-year follow-up study in the FIN-RACo trial)
STUDY POPULATIONS AND METHODS

Figure 3. Flowchart of study I.

Cross-sectional study in 1988

Nephropathy patients
n=103

HU n=54

PU n=27

HUPU n=7

Tot CRF n=29

Controls
n=102

Follow-up study in 2003

Deceased n=58

Attended n=29

Alive and non-attending n=16

Deceased n=50

Attended n=43

Alive and non-attending n=9

HU=isolated hematuria.
PU=isolated proteinuria
HUPU=combined hematuria and proteinuria
Tot CRF=chronic renal failure combined with or without hematuria and/or proteinuria
1. Populations

1.1 Study I

The population in study I was based on a cohort of RA patients living in the city of Tampere in 1987. According to the register of the Social Insurance Institute of Finland, a total of 1,051 (834 females, 217 males) could be confirmed as suffering from definite or classic RA according to the diagnostic criteria of the American Rheumatism Association (Ropes et al. 1958). These subjects were invited in 1987 to participate in a prospective study of renal and urinary tract diseases in patients with RA (Korpela 1993); 604 subjects (470 females, 134 males) participated in the study conducted in 1988. At that time the mean age of the RA patients was 59 ± 13 (mean ± SD) years and the mean duration of RA 15 ± 10 years.

Abnormal clinical renal findings were recorded in 103 out of the 604 RA patients (17%, nephropathy patients, NP), including isolated hematuria in 54 (9%), isolated proteinuria in 27 (5%), combined hematuria and proteinuria in 7 (1%), isolated chronic renal failure without hematuria or proteinuria in 15 (3%) and confirmed chronic renal failure combined with or without hematuria and proteinuria in 29 (5%) patients. Controls matched for age, sex and duration of RA were selected from among RA patients yielding no clinical renal findings (i.e. normal serum creatinine and urinalysis) (Figure 3). Further investigations of the NP group yielded 13 patients with definite or probable renal amyloidosis. In 2003, a follow-up study was made of the 103 NP patients and 102 controls. Seventy-two of these 205 RA patients attended and the 133 non-attenders were studied by evaluation of patient records (Figure 3).
1.2 Study II

The population in study II was based on the same prospective study of renal and urinary tract diseases in patients with RA as in study I (Korpela 1993). In 1988, detailed investigations of the 103 patients with NP findings yielded 17 with mesangial glomerulonephritis (MesGN). In 2003, 8 out of these 17 attended the follow-up study and 9 were studied through hospital records.

1.3 Study III

The study population here comprised 64 RA patients (47 women and 17 men) with diverse body composition and assumed renal function. All of them fulfilled the American College of Rheumatology 1987 criteria for RA (Arnett et al. 1988) and RF was positive in 44 of them. The age of the patients was 66 ± 11 (mean ± SD) years (range 41-86) and the duration of RA 21 ± 13 years (range 0-49). The body mass index (BMI) was 25 ± 5 kg/m$^2$ (range 15-34). The glucocorticoid dosing was 10 mg prednisolone or less in 51 patients and 15-20 mg in seven, while six were not on glucocorticoid therapy.

1.4 Study IV

This study population comprised of a cohort of 195 DMARD and glucocorticoid-naïve patients with recent-onset RA (duration of symptoms < 2 years) enrolled in the FIN-RACo Trial from April 1993 to May 1999 (Möttönen et al. 1999). The original trial was a multicenter, randomized, open parallel-group study and the inclusion criteria for entry were as follows: 1) fulfilment of the American College of Rheumatology 1987 revised criteria for RA (Arnett et al. 1988), 2) age 18-65 years, 3) duration of symptoms < 2 years, and 4) active disease with ≥3 swollen joints and at least 3 of the following: a)
ESR > 28 mm/hour or CRP > 19 mg/liter, b) morning stiffness of ≥29 minutes, c) > 5 swollen joints, or d) > 10 tender joints.

The FIN-RACo Trial was designed to compare two different DMARD treatment strategies. Patients were randomized to receive either combination DMARD therapy (COMBI, n=97) including sulfasalazine, methotrexate, hydroxychloroquine, and prednisolone, or single DMARD therapy (SINGLE, n= 98), initially with sulfasalazine, with or without prednisolone. Oral prednisolone was prescribed for 63 patients in the SINGLE group (according to treating clinicians’ decisions). The treatment was targeted to remission in all patients. If any of the components of combination treatment had to be discontinued, the combination of 3 DMARDs was rebuilt by replacing sulphasalazine and hydroxychloroquine with auranofin, and methotrexate with azathioprine. Other DMARDs could also be used as substitutes. In the SINGLE group sulphasalazine could be replaced by methotrexate and thereafter by auranofin, hydroxychloroquine etc., but only one DMARD at a time was allowed. After two years, the choice and dosing of DMARDs and prednisolone were not restricted, but the treatment was still aimed to achieve or maintain remission (Korpela et al. 2004; Rantalaiho et al. 2009). Between 2 and 11 years, combination DMARD therapy (at least two DMARDs at the same time) was used in 79% (median, IQR 43, 100) of the length of the follow-up period in the original COMBI group and in 54% (median, IQR 3, 94) in the original SINGLE group. The corresponding proportions of single DMARD therapy were 5% (median, IQR 0, 30) in the COMBI group and 35% (median, IQR 3, 67) in SINGLE (Rantalaiho et al. 2009).

One hundred and seventy-eight patients completed the two-year follow-up, 160 completed the 5-year follow-up and 138 participated in the 11-year check-up visit (Rantalaiho et al. 2009).
2. Methods

2.1 Determination of abnormal clinical findings and renal diseases in the cross-sectional study in 1988 (studies I and II)

The original RA population (Korpela 1993) was screened by first morning urine sample, 8-hour urine collection, and concomitant blood sample. If urinary albumin excretion was 150mg/8h or more or the urine albumin dipstick was positive, a diurnal urine collection was performed. Proteinuria was defined as urine protein excretion of 150 mg/24h or more and hematuria as a positive dipstick result in two consecutive urine samples. CRF was defined as serum creatinine 100 μmol/l or more in females and 115 μmol/l or more in males in two consecutive samples. Renal needle biopsy was considered if hematuria was constant and no urological lesion could be found, or proteinuria was 500mg/24h or more and there was no contraindication for biopsy.

Histological diagnosis of MesGN was made if abnormal findings were detected by at least two out of three methods of investigation: I) mild mesangial alterations consisting of increased matrix and/or hypercellularity in light microscopy, II) finely granular mesangial deposits of immunoglobulin with or without associated C3 in immunofluorescence microscopy, and III) electron-dense deposits located in the mesangial area between basement membrane and mesangial cell cytoplasm (Helin et al. 1986). Definite renal amyloidosis was diagnosed if amyloid deposits were found in histological examination of kidney biopsy specimens. Probable renal amyloidosis was established if the patient evinced clinical signs of renal disease or reduced renal function, and amyloidosis was proven histologically by biopsy of extrarenal organs, and the patient in question had no disease (e.g. hypertension, diabetes) or medication (e.g. gold salts or DPA) known to induce proteinuria.
2.2 Assessment of the prognosis of clinical and histologically proven renal disease and new abnormal renal findings based on the 13-year follow-up in patients with advanced RA (studies I and II)

In 2003 a follow-up study was made of the 103 NP patients and 102 controls (Figure 3). Patients attending (n=72, including 8 patients with MesGN from study II) underwent a detailed physical examination. The first morning urine sample was studied by test strip (Combur-10M-test, Roche Diagnostics). Assay sensitivities were 10 cells/µl for erythrocytes and 0.2 g/l for albumin. The differential particle count was studied by supravital staining of centrifuged urine sediments and microscopy. Urine bacterial culture was also performed. Renal function was measured by plasma creatinine (enzymatic colorimetric method) and by 24-hour creatinine clearance related to 1.73 m² of body surface area (normally ≥ 1.4 ml/s/1.73 m²):

\[
\left\{ \frac{V(\text{ml})}{86400\text{s}} \right\} \times \left\{ \frac{\text{urine creatinine} (\mu\text{mol/l}) \times 1.73/A}{\text{plasma creatinine} (\mu\text{mol/l})} \right\}
\]

where V= the 24 hour volume of urine (ml), and A= body surface area (m²)

Hematuria, proteinuria and CRF were defined as in the original cross-sectional study in 1988. Urine samples with pyuria, bacteriuria or urinary tract infection were excluded when estimating the occurrence of hematuria or proteinuria. If any of the abnormal findings was detected for the first time (since 1988), a renal ultrasound survey was undertaken. In the case of hematuria urine cytology and urethrocystoscopy were also carried out.

Specimens of subcutaneous abdominal fat obtained by fine-needle aspiration biopsy were studied by regular light microscopy and polarized microscopy after staining with Congo red (Westermark and Stenkvist 1973).

Clinical data on the patients not attending the study (n=133, including 9 patients with MesGN from study II) were gathered by evaluating the appropriate patient records in Tampere University Hospital and Tampere City Hospital. The latest available serum creatinine levels and urinalysis, and the
age of the patient at time of follow-up were recorded. A patient was defined as having proteinuria or hematuria if the latest urinary test strip for albumin or erythrocytes was positive. Urine samples with pyuria, bacteruria or urinary tract infections were excluded when estimating the occurrence of hematuria or proteinuria. A record was kept of data on tissue samples to detect amyloid, diagnosed renal diseases, hypertension and diabetes. A temporary elevation of serum creatinine was not regarded as a sign of reduced renal function. Treatments for end-stage renal failure, e.g. dialysis and renal transplantation, were recorded.

2.3 Evaluation of the diagnostic accuracy of various methods to estimate GFR in RA patients (study III)

In study III laboratory determinations were made in conjunction with a routine follow-up appointment in the outpatient ward of the Departments of Nephrology or Rheumatology in Tampere University Hospital. Plasma $^{51}$Cr-EDTA clearance was assessed by the single injection method and blood samples drawn at 0, 90 and 180 min (Garnett et al. 1967). A part of 0-min fasting sample was used to determine plasma creatinine, cystatin C and urea. Also 24-hour timed urine collections were obtained to determine endogenous creatinine clearance related to 1.73 m² of body surface area.

Plasma cystatin C and serum RF were measured immunoturbidimetrically on a Hitachi 704 analyser or on a Cobas Mira and Integra instrument (F.Hoffman-La Roche Ltd, Basel, Swizerland). Plasma creatinine was determined by the enzymatic colorimetric method using CREP2 (Roche Diagnostics) as reagent, urea by kinetic test with urease and glutamate dehydrogenase using Cobas Integra Urea/BUN (Roche Diagnostics) as reagent on the same instruments, respectively. The reference values for plasma creatinine were below 95 µmol/l for females and below 105 µmol/l for males. The reference values for plasma cystatin C were below 1.2 mg/l for individuals ≤ 50 years, below 1.4 mg/l
for age > 50 years. The reference values for urea were below 6.4 mmol/l for females <50 years, below 7.9 mmol/l for females ≥50 years, and below 8.1 mmol/l for males.

In addition, GFR was estimated according to the Cockcroft-Gault (CG) (Cockcroft and Gault 1976) formula and the Modification of Diet in Renal Disease (MDRD)(Levey et al. 2000) formula.

The possible influence of RF on plasma cystatin C values were studied by converting the plasma cystatin C values expressed as mg/l to estimated GFR (ml/min) using the following formula (Larsson et al. 2004): 94.652×plasma cystatin C^{-1.2478}. Thereafter the estimated GFR was reduced by the GFR measured by plasma $^{51}$Cr-EDTA clearance and the difference correlated to the concentration of RF in the sample.

2.4 Evaluation of the cumulative incidence of abnormal clinical renal findings and the influence of treatment strategies on findings in patients with early RA (study IV)

In study IV the serum creatinine and urine samples were analyzed at baseline and at months 6, 9, 12, 18, 24 and at yearly intervals thereafter up to 11 years. Hematuria and proteinuria were defined as positive by dipstick test. Assay sensitivities were defined according to the reference values of the local hospital. Renal function was likewise assessed by measuring serum creatinine concentration according the in-house method of each hospital. A raised serum creatinine concentration was defined as ≥100 µmol/l in females and ≥115 µmol/l in males. In addition, GFR was estimated according to the CG formula (Cockcroft and Gault 1976). A reduction in the estimated GFR below 60 ml/min/1.73m$^2$ was considered clinically significant.

Renal findings were defined as repeatedly abnormal if detected ≥ 3 times during the follow-up and as a single abnormality when it occurred at least once. In addition to the data concerning abnormal renal findings, the details of reported serious adverse events were also collected and recorded.
2.5 Statistical analyses

Unpaired comparisons were carried out using the Mann-Whitney U-test (two groups, skew-distributed) and the Kruskal-Wallis test (several groups). The data were compared to baseline using Wilcoxon’s signed-rank test for paired data. Categorical data were analyzed by $\chi^2$ test, Fisher’s exact test or McNemar test.

In study III the Pearson correlation coefficient was used for correlation analysis of the measured parameters (plasma cystatin C, creatinine, creatinine clearance, urea and estimated GFR using CG and MDRD formulas) with plasma $^{51}$Cr-EDTA clearance. Comparison of the differences between the correlation coefficients obtained was made by Statistica version 7.0 for Windows using the module- Difference Tests. Differences between plasma $^{51}$Cr-EDTA clearance and the CG estimates for GFR and plasma $^{51}$Cr-EDTA clearance and creatinine clearance were also studied using Bland-Altman plots (Bland and Altman 1986). The partial correlation test was used after adjusting for the level of serum RF, CRP and ESR to study the possible effect of these variables on correlation coefficients between $^{51}$Cr-EDTA and cystatin C.

In study IV all the RA patients who started DMARD therapy (n=195) were included. Last-observation carried-forward (LOCF) analysis was used when the clinical renal findings for each patient were analyzed. The Kaplan-Meier method was used to estimate the cumulative incidence of patients with clinical renal findings. Cox's regression analyses were made to adjust for confounding factors (age and sex). Also the prevalences of hematuria, proteinuria and clinically significant renal functional impairments were analyzed cross-sectionally during follow-up, and the analyses were based on time-by-time analysis. Computation was carried out using SPSS for Windows statistical software version 9.0.
3. Ethical considerations

All studies were carried out in compliance with the Helsinki Declaration. Approvals for studies I-III were obtained from the Ethical Committee of Tampere University Hospital and for study IV from the national health authorities and ethics committees in all 18 participating hospitals.

All patients gave written informed consent.
RESULTS

1. Demographics and details of treatments in the advanced and early RA study populations

The flowchart of RA patients with nephropathy (n=103) and the age- and sex-matched controls (n=102) are shown in Figure 3 (study I, page 48). The demographics of the study population are shown in Table 3. The median age in the NP group participating in the follow-up was lower than that of NP patients studied by hospital records: 69 years versus 74 years (p=0.006 for difference) and the situation was similar in the controls: 71 years versus 79 years (p<0.001 for the difference). The median follow-up time in the urine and serum creatinine sampling was 13 (range 0-16) years.

There was no statistical difference between the NP and the control group in the use of DMARD or glucocorticoid therapies except for azatioprine, which had been used more frequently in the NP group compared to the controls (23% vs. 7%, p=0.002). Patients who were or had been on azathioprine treatment had more frequently definite or probable renal amyloidosis than the rest of the patients (43% versus 6%, p< 0.001 for difference) and CRF was also detected more frequently in them (43% versus 24%, p=0.022 for difference).

The baseline demographics of the early RA study population are seen in Table 4. The medications used between 2 and 11 years are described in the section “Populations, study IV” (1.3).
Table 3. Demographics of RA patients in the original nephropathy (NP) and control groups in the cross-sectional study in 1988 and in the follow-up 2003 in study I

<table>
<thead>
<tr>
<th>Demographics of RA patients</th>
<th>Original group</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>103 (77)</td>
<td>102 (75)</td>
</tr>
<tr>
<td>Age in years, median (range)</td>
<td>63 (31-90)</td>
<td>62 (25-90)</td>
</tr>
<tr>
<td>Duration of RA in years, median (range)</td>
<td>16 (1-53)</td>
<td>14 (2-45)</td>
</tr>
</tbody>
</table>

Cross-sectional study in 1988:

Participants

Number of patients | 29 | 43
Age in years, median (range) | 69 (47-83) | 71 (40-82)
Duration of RA in years, median (range) | 26 (17-55) | 27 (16-54)

Non-participants

Number of patients (female) | 74 (54) | 59 (40)
Age in years, median (range) | 74 (53-94) | 79 (47-100)
Duration of RA in years, median (range) | 25 (4-54) | 23 (9-56)

Participants= RA patients studied prospectively in 2003
Non-participants= RA patients studied retrospectively in 2003
Table 4. The baseline demographics of the early RA study population in study IV

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Treatment group</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COMBI (n=97)</td>
<td>SINGLE (n=98)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female no. (%)</td>
<td>56 (58)</td>
<td>65 (66)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range) age</td>
<td>47 (23-65)</td>
<td>48 (20-65)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range) duration of disease (months)</td>
<td>7.3 (2-22)</td>
<td>8.6 (2-23)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

COMBI = combination DMARD therapy
SINGLE = single DMARD therapy

Table 5. The occurrence and persistency of abnormal clinical renal findings in the follow-up study of the advanced RA population (study I)

<table>
<thead>
<tr>
<th>Findings in the follow-up study in 2003</th>
<th>PU or Isolated NP-findings</th>
<th>CRF in total(%)</th>
<th>Normal findings (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline findings in 1988</td>
<td>HU</td>
<td>HUPU</td>
<td>CRF</td>
</tr>
<tr>
<td>RA patients with nephropathy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HU (n=54)</td>
<td>15</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>PU (n=27)</td>
<td>4</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>HUPU (n=7)</td>
<td>0</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>CRFisol (n=15)</td>
<td>1</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>CRF in total (n=29)</td>
<td>2</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Total (n=103)</td>
<td>20</td>
<td>30</td>
<td>19</td>
</tr>
<tr>
<td>RA patients with normal serum creatinine and urianalysis, controls (n=102)</td>
<td>5</td>
<td>13</td>
<td>11</td>
</tr>
</tbody>
</table>

HU=isolated hematuria,
PU=isolated proteinuria
HUPU=combined hematuria and proteinuria
CRFisol=isolated chronic renal failure without hematuria or proteinuria
CRF in total=chronic renal failure combined with or without hematuria and/or proteinuria, includes patients belonging also to PU, HU, HUPU, CRFisol groups
2. Proteinuria

2.1 Incidence of proteinuria in advanced RA

Isolated proteinuria or combined proteinuria and hematuria emerged in the follow-up in 13 out of the 102 control RA patients originally evincing normal serum creatinine and urinalysis results (Table 5). One of the patients had definite or probable renal amyloidosis and another had CRF (serum creatinine 128 µmol/l). Diurnal protein excretion ranged from 150 to 340 mg, and additionally the latest urinary test strip was positive in four patients.

2.2 Persistence and prognosis of proteinuria in advanced RA

As seen in Table 5, proteinuria persisted in the follow-up in 56% of the patients evincing proteinuria in 1988 if proteinuria was defined to comprise both isolated proteinuria and proteinuria combined with hematuria. The prevalence of definite or probable renal amyloidosis in 2003 was 41% in the original isolated proteinuria group and 71% in the combined proteinuria and hematuria group. In total, proteinuria was detected in 30 out of the 103 NP patients in 2003, and 13 of these (43%) had definite or probable renal amyloidosis. The prevalence of CRF in patients evincing proteinuria in the cross-sectional study in 1988 was 38%, 59% in 2003, and serum creatinine exceeded 200 µmol/l in 12 out of all 34 (35%) patients originally evincing proteinuria. Dialysis therapy was given to 10 out of 103 NP patients, 9 of whom belonged to the isolated proteinuria or the combined proteinuria and hematuria groups in the cross-sectional study.
2.3 Incidence of proteinuria in early RA

The cumulative incidences of repeated proteinuria in the COMBI and SINGLE groups during the two- and 11-year follow-up periods were 3.4% (95%CI 1.1-10.1) and 4.8% (95%CI 1.8-12.2) versus 1.1% (95%CI 0.2-7.8) and 5.3% (95%CI 2.0-13.7), respectively (p=0.93, adjusted for age and sex, Figure 4). Nor were statistical differences found between the cumulative incidences of the first single proteinuria findings; during the two- and 11-year follow-up periods in the COMBI and SINGLE groups the incidences were 17.9% (95%CI 11.1-27.2) and 30.9% (95%CI 22.4-41.6) versus 11.3% (95%CI 6.4-19.5) and 27.9% (95%CI 19.6-38.9), respectively (p=0.43, adjusted for age and sex). The cross-sectional prevalence of proteinuria fluctuated from visit to visit, but no difference between treatment strategy groups could be found (Table 6).

During the whole 11-year follow-up period proteinuria was detected 55 times and only one of the findings (in the SINGLE group) was related to the use of DPA. The proteinuria in the patient in question progressed to nephrotic-range proteinuria. Renal biopsy indicated a histological diagnosis of minimal-change nephropathy. Proteinuria resolved totally within a couple of months after the cessation of DPA. None of the proteinuria findings was related to the use of gold salts.
Figure 4. Cumulative incidence of repeated proteinuria in combination (COMBI) and single (SINGLE) treatment groups (study IV)

Table 6. Cross-sectional (time-by-time analysis) prevalences (number of patients, %) of abnormal clinical renal findings among patients attending follow-up visits in study IV.

<table>
<thead>
<tr>
<th>Finding</th>
<th>COMBI</th>
<th>SINGLE</th>
<th>COMBI</th>
<th>SINGLE</th>
<th>COMBI</th>
<th>SINGLE</th>
<th>COMBI</th>
<th>SINGLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinuria</td>
<td>1/97</td>
<td>3/98</td>
<td>7/92</td>
<td>3/95</td>
<td>1/82</td>
<td>0/85</td>
<td>3/77</td>
<td>4/73</td>
</tr>
<tr>
<td>Hematuria</td>
<td>10/97</td>
<td>8/98</td>
<td>8/92</td>
<td>11/95</td>
<td>9/82</td>
<td>10/85</td>
<td>8/77</td>
<td>7/73</td>
</tr>
<tr>
<td>Elevated serum creatinine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2/97</td>
<td>1/98</td>
<td>2/92</td>
<td>0/95</td>
<td>3/82</td>
<td>3/85</td>
<td>2/77</td>
<td>1/73</td>
</tr>
<tr>
<td>eGFR&lt;sub&gt;CG&lt;/sub&gt; &lt;60 ml/min&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5/97</td>
<td>1/98</td>
<td>3/92</td>
<td>3/95</td>
<td>6/82</td>
<td>5/85</td>
<td>4/77</td>
<td>5/73</td>
</tr>
</tbody>
</table>

<sup>a</sup>defined as 100 µmol/l or more in females and 115 µmol/l or more in males

<sup>b</sup>glomerular filtration rate estimated by Cockcroft-Gault formula
3. Hematuria

3.1 Incidence of hematuria in advanced RA

Table 5 shows that isolated hematuria emerged in 5 patients in the original control group. None of these new hematuria patients had definite or probable renal amyloidosis. Two of the five had CRF (serum creatinine of 125-247 µmol/l). The sole urological malignancy (bladder cancer) found in this study was diagnosed in a control patient with combined hematuria, proteinuria and CRF.

3.2 Persistence and prognosis of hematuria in advanced RA

Isolated hematuria continued in 28% of the original isolated hematuria patients (Table 5) and no subject in the persisting isolated hematuria group had definite or probable renal amyloidosis. Six percent of the total isolated hematuria group (n=54) evinced definite or probable renal amyloidosis and dialysis therapy was given to one of them. CRF was seen in one out of 54 isolated hematuria patients at baseline and CRF emerged in 20% of them in the follow-up. Serum creatinine exceeded 200 µmol/l in 4 patients (8%). Totally normal findings were recorded in one third. None of the whole NP group evinced a urological cause for hematuria in the follow-up.
3.3 Incidence of hematuria in early RA

The cumulative incidences of repeated hematuria in the COMBI and SINGLE groups during the two- and 11-year follow-up periods were 9.4% (95% CI 4.8-18.0) and 14.1% (95%CI 8.0-24.2) versus 16.4% (95% CI 10.0-26.1) and 22.1% (95%CI 14.5-33.0), respectively (p=0.14, adjusted for age and sex, Figure 5). If the cumulative incidences were analyzed according to the first single hematuria finding, a statistical difference was seen between the incidences in COMBI and SINGLE: 17.7% (95%CI 15.7-20.0) versus 27.0% (95%CI 24.6-29.5) during the two-year follow-up period, respectively, while the corresponding figures during the 11 years’ follow-up were 38.4% (35.6-41.3) versus 45.5% (42.6-48.4) (adjusted p< 0.001, Figure 6). The cross-sectional prevalence of hematuria was fairly stable in both treatment groups throughout the 11-year follow-up (8-12%) (Table 6).

Figure 5. Cumulative incidence of repeated hematuria in combination (COMBI) and single (SINGLE) treatment groups (study IV)
4. Chronic renal failure (CRF)

4.1 Incidence of chronic renal failure in advanced RA

CRF was detected in 14 out of the 102 control RA patients by the follow-up in 2003 and most of the findings were isolated (Table 5). Hypertension was more frequent in control patients with CRF than without (79% versus 44%, p= 0.015). Serum creatinine exceeded 200 µmol/l in 4 patients and all of these had definite or probable renal amyloidosis. Dialysis therapy was given to two control patients with isolated CRF and histologically confirmed amyloidosis.
4.2 Persistence and prognosis of chronic renal failure in advanced RA

CRF continued in 53% out of the 15 original isolated CRF patients in the follow-up and the findings remained isolated in all cases (Table 5). One patient (7%) in the original group had definite or probable renal amyloidosis, and the clinical follow-up finding was also isolated CRF. None of the patients was given dialysis therapy and the serum creatinine level also remained below 200 µmol/l in all. If CRF patients with combined proteinuria and/or hematuria (Total CRF, n=29) were included in the analysis, the CRF finding was continual in 66% and serum creatinine exceeded 200 µmol/l in 28% of the patients originally belonging to total CRF group. Twenty-one per cent of the patients were given dialysis therapy and definite or probable renal amyloidosis was found in 38% of the total CRF patients.

4.3 Incidence of chronic renal failure in early RA

The cumulative incidences of repeatedly raised serum creatinine in the COMBI and SINGLE groups during the two- and 11-year follow-up periods were 2.1% (95%CI 0.5-8.0) and 4.4% (95%CI 1.7-11.4) versus 4.2% (95%CI 1.6-10.7) and 6.7% (95%CI 3.0-14.3) (p= 0.87, adjusted for age and sex, Figure 7). The corresponding cumulative figures of the eGFR<sub>GC</sub> < 60 ml/min/1.73m² in COMBI and SINGLE were 7.3% (95%CI 3.5-14.7) and 11.9% (95%CI 6.8-20.5) versus 9.3% (95%CI 5.0-17.1) and 10.5% (95%CI 5.8-18.7), respectively (adjusted p=0.85, Figure 8). If cumulative incidences of the first raised creatinine finding were analyzed in COMBI and SINGLE during the two- and 11-year follow-up periods, the figures were 6.3% (95%CI 2.9-13.4) and 9.7% (95%CI 5.6-18.2) versus 10.2% (95%CI 5.6-18.2) and 16.1 % (95%CI 10.0-25.4), respectively (adjusted p= 0.38), and those of eGFR<sub>GC</sub> < 60 ml/min/1.73m² 8.3% (95%CI 4.2-15.9) and 15.1% (95%CI 9.2-24.3) versus 11.3% (95%CI 6.4-19.5)
and 17.5% (95%CI 11.1-27.1). The cross-sectional prevalence of elevated serum creatinine values was 2-4% and that of glomerular filtration rates < 60 ml/min 1-7% throughout the study in both groups (Table 6).

During the whole follow-up, serum creatinine was raised in 90 measurements and the finding was associated with cyclosporine use in three cases. eGFR<sub>GC</sub> < 60 ml/min/1.73m<sup>2</sup> was found in 272 measurements, but only three of the findings were detected during cyclosporine use.

Figure 7. Cumulative incidences of repeated elevated serum creatinine findings in combination (COMBI) and single (SINGLE) treatment groups (study IV)
5. Long-term prognosis of renal diseases

In the advanced RA population (studies I and II), renal needle biopsy was taken in 38 of the 103 original NP cases (37%) up to the end of the follow-up in 2003. Among the 31 representative samples, histological findings were MesGN in 17 patients, amyloidosis in 8, membranous glomerulonephritis in two, diabetic glomerulosclerosis in two, interstitial nephritis and hyalinic atherosclerosis and normal finding in one patient each. In RA controls, renal needle biopsy was undertaken for two out of 102 patients. Amyloid was found in one of the samples.
5.1 Renal AA-amyloidosis

The available tissue samples for detection of amyloid in the NP group during the cross-sectional study in 1988 and up to 2003 are demonstrated in Table 7. Also shown are the proportion of positive findings and the sites of them. Up to the end of the follow-up, 8 cases of histologically proven renal amyloidosis were established and 2 additional cases emerged in post-mortem histological studies.

All except one of the 20 definite or probable renal amyloidosis patients in the NP group had CRF in the follow-up. Proteinuria was seen in 8, hematuria in one, hematuria combined with proteinuria in 5 and isolated CRF in 6. Serum creatinine exceeded 200 µmol/l in 15 out of the 20 (75%) patients, dialysis therapy was given to 9 (45%), and renal transplantation was performed for one.

Table 7. Data on tissue samples obtained to detect amyloid in NP group in the baseline study in 1988 and in the follow-up in 2003 (study I)

<table>
<thead>
<tr>
<th></th>
<th>RA patients, NP group (N=103)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline in 1988</td>
</tr>
<tr>
<td>Tissue samples to detect amyloid available</td>
<td>96/103 (93%)</td>
</tr>
<tr>
<td>Site of the positive tissue samples:</td>
<td></td>
</tr>
<tr>
<td>subcutaneous tissue</td>
<td>11</td>
</tr>
<tr>
<td>kidney</td>
<td>6</td>
</tr>
<tr>
<td>gastrointestinal tract</td>
<td>5</td>
</tr>
<tr>
<td>Patients yielding positive results in any of the available samples to detect amyloid</td>
<td>18/96 (19%)</td>
</tr>
<tr>
<td>Definite or probable renal amyloidosis</td>
<td>13/103 (13%)</td>
</tr>
</tbody>
</table>
In the baseline study in 1988, tissue samples for detection of amyloid were not obtained systemically from the control group, samples being thus available for only four out of the 102 control RA patients. Up to 2003 tissue samples were available for 64 patients (63%) in the follow-up, and 13 of these (20%) showed amyloid deposits in histological examination. There was one patient with histologically confirmed renal amyloidosis and three patients with probable renal amyloidosis. Three of these four patients had isolated CRF (serum creatinine exceeding 200 µmol/l in all) and one patient had isolated proteinuria. Dialysis therapy was given to two controls.

5.2 Mesangial glomerulonephritis

In 2003, a follow-up study was made of the 17 MesGN patients diagnosed during the original cross-sectional study in 1988. The median follow-up time for urine and serum creatinine sampling was 15 (range 2-16) years. The baseline and follow-up findings are shown in Table 8.

Two MesGN patients evinced abnormal serum creatinine. Renal biopsy had been performed twice for the patient with a serum creatinine level of 221 µmol/l. The histological finding was MesGN in the first sample in 1988 and renal amyloidosis in the second, obtained seven years later. In the other patient with a serum creatinine level of 174 µmol/l damage was seen in her urinary collecting system after radiotherapy for uterine cervical carcinoma.

MesGN-associated proteinuria was mild, with the exception of one patient (urine protein excretion 1.4 g/24h), who also yielded amyloid deposits in his subcutaneous fat aspiration specimen. Altogether, positive findings were recorded in amyloid samples in four of the 17 patients (one renal and three extrarenal) and two of these patients had probable or definite renal amyloidosis, while two other patients yielded no clinical renal findings related to positive extrarenal amyloid samples.
Table 8. Occurrence of abnormal clinical renal findings in RA patients with mesangial glomerulonephritis (MesGN) in the baseline study in 1988 and in the follow-up study in 2003 (study II)

<table>
<thead>
<tr>
<th>Nephropathy finding</th>
<th>Baseline in 1988</th>
<th>Follow-up in 2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated hematuria</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Isolated proteinuria</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Combined hematuria and proteinuria</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Isolated chronic renal failure</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Normal</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>

6. Diagnostic accuracy of measurements of renal function in RA patients

The measurements of plasma cystatin C, creatinine, urea, creatinine clearance and creatinine-based formulas were compared to establish the most accurate means of assessing GFR in RA patients. $^{51}$Cr-EDTA clearance served as reference method. All the parameters studied are shown in Table 9. Four of the patients showed normal GFR ($\geq$90 ml/min/1.73m$^2$) while the remainder fell into the category of mildly to severely decreased kidney function; stage 2-4 (GFR 15-89 ml/min/1.73m$^2$).
Table 9. The parameters representing kidney function in the study group (study III)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>median</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{51}$Cr EDTA (ml/min/1.73m$^2$)</td>
<td>44</td>
<td>17-135</td>
</tr>
<tr>
<td>Plasma cystatin C (mg/l)</td>
<td>1.50</td>
<td>0.62-4.65</td>
</tr>
<tr>
<td>Plasma urea mmol/l</td>
<td>8.70</td>
<td>4.00-31.90</td>
</tr>
<tr>
<td>Plasma creatinine (µmol/l)</td>
<td>104</td>
<td>39-475</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min/1.73m$^2$)</td>
<td>47</td>
<td>8-145</td>
</tr>
<tr>
<td>GFR$_{MDRD}$ (ml/min/1.73m$^2$)</td>
<td>54</td>
<td>6-149</td>
</tr>
<tr>
<td>GFR$_{CG}$ (ml/min/1.73m$^2$)</td>
<td>53</td>
<td>9-156</td>
</tr>
</tbody>
</table>

$^{51}$Cr EDTA = plasma $^{51}$Cr EDTA clearance  
GFR$_{MDRD}$ = GFR estimated by Modification of Diet in Renal Disease formula  
GFR$_{CG}$ = GFR estimated by Cockcroft-Gault formula

The sensitivity of the tests to identify patients with reduced GFR is shown in Table 10. When plasma $^{51}$Cr-EDTA clearance falls below 90 and 60 ml/min/1.73m$^2$, plasma creatinine still remains within normal range in 42% and 19% of the patients. Plasma cystatin C remained within normal range in 29% and 12% of the patients with stage 2 (GFR<90ml/min) or stage 3 (GFR<60ml/min) CKD, respectively, while the CG and MDRD estimates for GFR and plasma creatinine clearance showed better sensitivity.
Table 10. Proportions of the GFR markers studied markers showing incorrectly normal values even while the directly measured kidney function is mildly or moderately impaired (study III)

<table>
<thead>
<tr>
<th>Test</th>
<th>$^{51}$Cr-EDTA &lt; 90 ml/min/1.73m²</th>
<th>$^{51}$Cr-EDTA &lt; 60 ml/min/1.73m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma creatinine⁴, n=64</td>
<td>25/60 (42)</td>
<td>8/43 (19)</td>
</tr>
<tr>
<td>normal/all (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma cystatin C⁵, n=62</td>
<td>17/58 (29)</td>
<td>5/42 (12)</td>
</tr>
<tr>
<td>normal/all (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFR$_{MDRD}^c$, n=64</td>
<td>6/60 (10)</td>
<td>0</td>
</tr>
<tr>
<td>≥90 ml/min/all (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFR$_{CG}^c$, n=64</td>
<td>7/60 (12)</td>
<td>1/43 (2)</td>
</tr>
<tr>
<td>Creatinine clearance, n=54</td>
<td>8/50 (16)</td>
<td>0</td>
</tr>
<tr>
<td>≥90 ml/min/all (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma urea⁶, n=45</td>
<td>15/41 (37)</td>
<td>3/27 (11)</td>
</tr>
<tr>
<td>normal/all (%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

⁴ Differentiation limit: 95 mmol/l for women, 105 mmol/l for men.

⁵ Two patients were missing cystatin C value. Differentiation limit: 1.20 mg/ml for individuals ≤50 years and 1.40 mg/ml for those > 50 years.

⁶ GFR estimated by MDRD and Cockcroft and Gault formulas.

⁶ Differentiation limits: 6.4 mmol/l for women <50 years and 7.9 mmol/l for women ≥ 50 years and 8.1 mmol/l for men.

The ability of the creatinine clearance, cystatin C (p< 0.0001 for both, $\chi^2$–test), plasma urea (p<0.001, $\chi^2$–test) and the MDRD (p=0.004, $\chi^2$–test) and CG formulas (p=0.017, $\chi^2$–test) to identify patients with decreased GFR (< 90ml/min, stage 2 CKD) was better than that of plasma creatinine. Likewise, creatinine clearance (p=0.001, $\chi^2$–test) and the MDRD and CG formulas (p=0.007 for both, $\chi^2$–test) proved superior in identifying patients with decreased GFR to plasma cystatin C. The ability of plasma urea to identify patients with stage 2 CKD was inferior to that of the creatinine clearance (p=0.002, $\chi^2$–test), CG and MDRD formulas (p= 0.001 for both, $\chi^2$-test).
All parameters were correlated to \( {^{51}}\text{Cr-EDTA} \) clearance and the correlation coefficients (Pearson) and the differences between the coefficients are shown in Table 11.

Table 11. Pearson correlation coefficients (CC) between the studied markers of GFR and plasma \( {^{51}}\text{Cr-EDTA} \) clearance

<table>
<thead>
<tr>
<th>Markers of GFR</th>
<th>CC</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma creatinine</td>
<td>0.800</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma cystatin C</td>
<td>0.863</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GFR\textsubscript{MDRD}</td>
<td>0.866</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GFR\textsubscript{CG}</td>
<td>0.904*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma creatinine clearance</td>
<td>0.922**</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma urea</td>
<td>0.817</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

GFR\textsubscript{MDRD} = GFR estimated by MDRD formula
GFR\textsubscript{CG} = GFR estimated by CG formula
*Correlation coefficient statistically better than that of creatinine or urea 
\( (p=0.0412 \) and \( p=0.0480 \), respectively) 
**Correlation coefficient statistically better than that of creatinine or urea 
\( (p=0.0099 \) and \( p=0.0176 \), respectively)

CRP was available for 42 patients and ESR for 38. The median of CRP was 8.8 mg/l (range 1.2-51.6 mg/l) and of ESR 25 mm/h (range 2-74). Partial correlation coefficients between plasma cystatin C and \( {^{51}}\text{Cr-EDTA} \) clearance after adjusting for RF, CRP or ESR did not differ significantly from the nonadjusted figures. Nor did the level of RF influence plasma cystatin C levels.
The level of GFR estimated by the GC and MDRD formulas appeared to be approximately 20% higher than that measured by plasma $^{51}$Cr-EDTA clearance. The divergence between the CG estimates and creatinine clearance increased at GFRs over 60 ml/min.
DISCUSSION

1. Incidence of abnormal clinical renal findings in advanced and early RA

During the 13-year follow-up of the advanced RA population in study I, new nephropathy findings were recorded in almost one third of the RA patients who were totally free out of them in the baseline study in 1988. The new findings were mostly mild in character. CRF (14%), proteinuria, or proteinuria combined with hematuria (13%) were the most frequent new findings, whilst isolated hematuria was discovered in only 5 out of the 102 original control RA patients. Four patients evinced more serious renal functional impairment and also probable or definite renal amyloidosis.

There are only a few prospective reports concerning the incidence of clinical renal findings in advanced RA patients, but these findings are mostly related to gold and DPA treatments. Also the methodology in these studies varies substantially from one to the other. However, the occurrence of isolated proteinuria in the present study is in agreement with figures (10.4%-11.7%) obtained from 5-year follow-up studies (Bourke et al. 1981; Cantagrel et al. 1990). The incidence of isolated hematuria has been higher in earlier follow-up-studies, ranging from 8-10% (White et al. 1984; Cantagrel et al. 1990) to even one third of the patients (Leonard et al. 1987) than in the present study. In the study by Leonard et al. (1987) the follow-up program was particularly intensive, which might have had an influence on the result. The incidence of isolated renal failure in Cantagrel’s (1990) follow-up study was evidently lower than in the present study, but the patient population in that study was younger and additionally, the cut-off point for serum creatinine was higher than in here.
In the follow-up study of early RA patients, the cumulative 11 year’s incidence of repeated proteinuria was around 5% and increased even six-fold if the analysis was made according to the first single finding. The figures for repeated proteinuria were slightly lower than those reported in one previous study of an early RA population (7%) with a median 3.5 years’ follow-up (Koseki et al. 2001). However, the proportion of intermittent findings in the present study was substantially higher than in the last-mentioned (Koseki et al. 2001). The persistent proteinuria in early RA patients has been linked mostly to DMARDs (DPA, gold salts, bucillamine) (Koseki et al. 2001), but in the present study only one of the proteinuria findings was evidently associated with the use of nephrotoxic DMARDs (DPA). The first-line DMARDs used in the FIN-RACo Trial did not include DMARDs known to induce proteinuria, especially gold salts and DPA, and there was no use of bucillamine at all in the present study.

In the early RA population (study IV) repeated hematuria was detected frequently (14-22%), and the cumulative incidence of the first single findings was even twofold, the results being more or less identical to those in an earlier report (Koseki et al. 2001). The cumulative occurrence of first single hematuria findings in the SINGLE group was greater than in the COMBI group, and a similar tendency was detected in the repeated findings. This may be related to earlier reports of higher disease activity in the SINGLE as compared to the COMBI group during the whole 11-year follow-up in the FIN-RACo study (Möttönen et al. 1999; Rantalaiho et al. 2009), and to the finding that isolated hematuria is associated with higher serum CRP levels in at least some patients with early RA (Koseki et al. 2001). A contribution of the histological finding of MesGN to a more frequent occurrence of isolated hematuria in patients with active RA can be suggested, as MesGN is considered to be an extra-articular manifestation of the basic rheumatoid disease (Korpela et al. 1997). However, undisputed data on the relationship are difficult to obtain, as isolated microscopic hematuria is not at present an indication for renal biopsy (Cohen and Brown 2003).
The cumulative incidence of repeatedly raised serum creatinine (4-7%) in the early RA population (study IV) was in line with figures in a previous report (Koseki et al. 2001), as were also the adopted criteria for raised serum creatinine. The occurrence of repeated findings of GFR estimated by the CG formula (eGFRCG) <60 ml/min/1.73m² was 11-12%. Both of these findings representing decreased renal function were associated with the use of cyclosporine only occasionally (in three pathological values in both). In the previous report mentioned, all medications used during the follow-up were recorded and most of the raised serum creatinine findings were related to therapy, however, only occasionally to DMARD therapy (Koseki et al. 2001).

In the FIN-RACo trial, with the exception of a more frequent occurrence of single hematuria in the SINGLE group, no other differences in clinical renal findings were detected according to treatment strategy. The renal safety profile of the initial combination DMARD treatment strategy was thus found favorable. The present study is to the author’s knowledge pioneering work in respect of the renal safety issues in combination DMARD therapy.

In conclusion, new abnormal clinical renal findings appearing later in the course of RA were common, but mild in character. Those who were free of clinical renal disease during the first 10-15 years after the onset of RA were not particularly prone to serious renal disease later on. In early RA patients the occurrence of clinical renal findings was in accord with previous observations, in which repeated hematuria was found to be common, whereas proteinuria and chronic renal failure were more infrequent findings. Furthermore, initial combination DMARD therapy with sulfasalazine, methotrexate, hydroxychloroquine and low-dose prednisolone in early RA patients was found to be safe for the kidneys and did not induce more nephrological complications than the traditional therapy with a single DMARD.
2. Prognosis and etiology of abnormal clinical renal findings

In the 13-year follow-up period, proteinuria persisted in over half of the original advanced RA patients with proteinuria, and renal function was impaired in over half of them. All but one of the dialysis patients in the NP group belonged originally to the proteinuria or combined proteinuria and hematuria groups. Increasing duration of RA and proteinuria combined to CRF has been found to increase the probability of AA-amyloidosis in RA patients with proteinuria (Korpela 1993; Helin et al. 1995; Nakano et al. 1998) as seen also in the present study. The prevalence of definite and probable renal amyloidosis was highest in the original proteinuria groups compared to other nephropathy subgroups, and its high prevalence largely explains the poor long-term prognosis of proteinuria here as elsewhere. In addition, the mortality rate among the RA patients with isolated proteinuria, combined proteinuria and hematuria, and microalbuminuria has been shown to be increased (Jacobsson et al. 1993; Sihvonen et al. 2004).

In the present follow-up of advanced RA patients, isolated hematuria showed a favorable renal prognosis, although the finding continued in almost one third. Isolated hematuria was less often associated with CRF than the other nephropathy subgroups, and the incidence of more severe renal functional impairment, as well as the need for the dialysis therapy, was infrequent. Isolated hematuria has not been regarded as a typical presenting finding in renal amyloidosis, as it usually combines with proteinuria (Brandt et al. 1968; Korpela 1993; Helin et al. 1995). In agreement with earlier reports, renal amyloidosis was found less frequently in the original isolated hematuria group than in the other nephropathy groups, which probably at least partly explains the good prognosis in the follow-up. Furthermore, the most frequent renal histological finding associated with isolated hematuria in RA
patients has been MesGN, associated with favorable renal functional prognosis (Hordon et al. 1984; Cantagrel et al. 1990; Korpela et al. 1995). A substantial proportion of the microscopic hematuria has proved to be transient in RA populations (Koseki et al. 2001) as well in normal (Froom et al. 1984). The etiology of isolated microhematuria remains in many cases unknown even after careful investigations (White et al. 1984; Korpela et al. 1995). The unspecific and transient nature of non-urological isolated hematuria may in part contribute to the good prognosis of the finding seen in the present study.

Isolated CRF continued in half of the original group and new other nephropathy findings were detected infrequently in this group. Serum creatinine exceeded 200 µmol/l in none of the patients and none needed dialysis therapy. The prevalence of definite or probable renal amyloidosis in the isolated CRF group was clearly lower than that in the total CRF group, which also included patients with proteinuria and/or hematuria. In the total CRF group the overall renal prognosis was poorer than in the isolated CRF patients. In kidney biopsy and post-mortem studies, arteriosclerosis and nephrosclerosis have been common findings in RA patients, especially in those over 50 years (Pollak et al. 1962; Pasternack et al. 1967; Boers et al. 1987). With advancing age the percentage of sclerotic glomeruli increases and tubulointerstitial fibrosis develops. These structural changes are accompanied by functional changes and GFR is known to decline slowly with age by 10 ml/min/1.73m$^2$ per decade after 30 years of age (Davies and Shock 1950). The decline in GFR with increasing age is largely attributable to hypertension (Lamb et al. 2003) and a relationship of age-related glomerulosclerosis and atherosclerosis has also been shown (Kasiske 1987). Accordingly, in the original population-based cross-sectional study in 1988, isolated CRF was related to ageing and hypertension probably explaining the good prognosis of the finding in the follow-up (Korpela 1993). If CRF is combined with proteinuria in patients with long-lasting RA, the probability of renal amyloidosis increases (Pollak et al. 1962; Boers et al. 1987; Korpela 1993; Helin et al. 1995; Nakano et al. 1998), as also seen here.
In conclusion, in patients with long-lasting RA isolated proteinuria and proteinuria combined with hematuria and/or CRF persisted very frequently in the long-term follow-up and these findings had poor clinical long-term prognosis mostly by reason of underlying renal amyloidosis. Isolated hematuria and isolated CRF were associated with evidently better prognosis with good preservation of renal function and infrequent need for dialysis therapy.

3. Prognosis of renal AA-amyloidosis and mesangial glomerulonephritis

With regard to the frequent appearance of serious CRF and also the frequent need for dialysis therapy, the long-term prognosis of renal amyloidosis was found to be unfavorable in the present study, this being in accord with previous observations (Browning et al. 1985; Hazenberg and van Rijswijk 1994; Joss et al. 2000). Joss et al. (2000) reported a median renal survival, i.e. patients alive and independent of renal replacement therapy, of 25 months from the diagnosis of renal amyloidosis in cases with chronic rheumatic diseases. Additionally, the mortality among the patients with histologically confirmed renal amyloidosis has previously been studied in the same RA patient population as in study I, and over two-fold mortality was detected compared to patients without nephropathy (Sihvonen et al. 2004). However, all these results reflect the effect of the DMARD strategies used from the 1960s to the mid-1990s, when the treatment of RA was based on the use of single DMARDs, the use of cytotoxic drugs was infrequent, and biologicals were not available at all. The prognosis has been shown to be markedly more favourable in patients with a sustained decrease in inflammation (Berglund et al. 1993; Gillmore et al. 2001; Nakamura et al. 2007).
Renal involvement is known to dominate the clinical course in patients with AA-amyloidosis (Gertz and Kyle 1991; Joss et al. 2000; Lachmann et al. 2007). This was the situation in the present study; most of the patients yielding positive amyloid samples also had evidence of definite or probable renal amyloidosis in the NP group. The latency between the onset of inflammation and clinical presentation with AA-amyloidosis has shown to be 16-18 years (Hazenberg and van Rijswijk 1994; Kobayashi et al. 1996; Gomez-Casanovas et al. 2001; Uda et al. 2006; Lachmann et al. 2007). In the present study, the long follow-up period allowed the appearance of clinical signs of amyloidosis in addition to e.g. positive results in subcutaneous biopsy. In the control RA group, amyloid sampling was not as extensive as in the NP group, but also in this group 20% of all available samples yielded positive results in 2003. Nevertheless, only 4 patients gave clinical or histological proof of renal amyloidosis reflecting the later appearance of amyloidosis in this group. Gomez-Casanovas et al. (2001) found that 16.3% of patients with RA of at least 5 years’ duration have amyloid deposits in their abdominal fat. They considered the prognostic value of positive subcutaneous biopsy questionable because at least 73% of the findings remained subclinical in the mean 6 years’ follow-up. However, the follow-up period was probably too short, as the patients with subclinical amyloidosis had shorter duration of RA than those with clinical disease.

The prognosis of MesGN was found favorable confirming the findings in two previous shorter follow-up studies (Kelly et al. 1988; Korpela et al. 1991). The development of CRF was not attributable solely to MesGN in any cases, as the two patients having CRF also showed other more likely reasons for impaired renal function. Previous studies have demonstrated that MesGN presenting with microscopic hematuria does not lead to deterioration in renal function (Kelly et al. 1988), while MesGN presenting with proteinuria is associated with poorer prognosis (Korpela et al. 1991). The poorer prognosis associated with the MesGN presenting with proteinuria was not confirmed in the
present study, since proteinuria was usually mild with the exception of a patient also suffering from probable renal amyloidosis, and proteinuria findings were not accompanied by CRF.

In conclusion, the good clinical prognosis of MesGN in RA patients was confirmed in the present median 15 years’ follow-up, as was the poor clinical prognosis of renal amyloidosis at least in patients treated with traditional DMARDs.

4. Usefulness of the estimates of GFR in detecting chronic renal failure in RA patients

Creatinine clearance and the CG formula were here found to have a significantly better correlation with GFR measured by $^{51}$Cr-EDTA clearance than plasma creatinine or urea (study III). Also these tests and the MDRD equation formula were superior in identifying RA patients with decreased GFR (<90ml/min/1.73m$^2$) to plasma cystatin C, creatinine or urea. Creatinine clearance has been regarded as a more sensitive marker of renal dysfunction than measurement of serum creatinine (Levey et al. 1988), but direct comparisons have not previously been made in RA patients. The correlation of creatinine clearance with an exogenous marker of GFR has been reasonable in RA patients (Richards et al. 1988; Boers et al. 1990). However, this requires timed urine collection, and furthermore, a significant day-to-day variation in creatinine excretion has been observed, consistent with incomplete collection (Goldberg and Finkelstein 1987). There have even been calls for its abandonment by laboratories and clinicians (Lamb et al. 2003), but the present results illustrate that creatinine clearance is still a useful measure of renal function in RA patients. Delicate guidance of patients, and the fact that most of them were experienced in urine collections, were probably related to the good diagnostic accuracy of the test.
Creatinine clearance is known to overestimate GFR by 10-20 ml/min/1.73m$^2$ by reason of tubular secretion of creatinine. Also in the present study the level of GFR measured by creatinine clearance was slightly higher than that measured by $^{51}$Cr-EDTA. The difference tended to grow as GFR decreased, since creatinine clearance overestimates lower GFRs.

The CG formula was found to constitute an adequate tool to detect reduced GFR in RA patients. This is in agreement with an earlier report on the good performance of the CG formula in RA patients with a correlation coefficient of 0.82 between the formula and directly measured GFR (Boers et al. 1994). The CG formula was originally developed to estimate creatinine clearance and most of the studies conducted among RA populations have focused on comparing the formula to creatinine clearance (Boers et al. 1988; Anders et al. 2000; Laiho et al. 2001; Anders et al. 2002). Judging from the literature, however, the CG formula is nowadays mostly applied to estimate GFR.

The CG formula and also the MDRD formula overestimated GFR by approximately 20%, which is in line with a previous report comparing CG to creatinine clearance (Boers et al. 1988). The accuracy of GFR-estimating equations is known to be impaired in persons with normal renal function. Also in the present study, the divergence in the CG and MDRD equations for the estimation of GFR values increased at measured GFRs over 60 ml/min. CG showed here only a slightly better correlation with $^{51}$Cr-EDTA clearance than did the MDRD formula, whereas the difference was clearer ($r=0.69$ versus 0.41) in a study by Anders et al. (2002), in which creatinine clearance served as reference method. In that study, the original MDRD formula containing urea and albumin was applied, whereas the abbreviated formula was used here.

In the present study, the correlation between $^{51}$Cr-EDTA clearance and plasma cystatin C was no better than that between $^{51}$Cr-EDTA clearance and plasma creatinine measurement. This is in contrast to earlier reports showing plasma cystatin C to correlate better with golden standard estimates of GFR than plasma creatinine, especially in patients with mild to moderate renal disease (Kyhse-
Andersen et al. 1994; Newman et al. 1994; Grubb 2000; Dharnidharka et al. 2002). Knowledge of the diagnostic accuracy of plasma cystatin C in RA patients is scant. Only one earlier study has been conducted among RA patients (Mangge et al. 2000), and it found plasma cystatin C to be better in detecting early renal impairment than plasma creatinine. However, creatinine clearance was used as reference method in that study. Also in the present study, plasma cystatin C identified the patients with decreased GFR (< 90 ml/min) slightly better than did plasma creatinine, but still the creatinine-based prediction equations and creatinine clearance proved superior to plasma cystatin C in detecting decreased GFR.

The serum concentration of cystatin C is determined mainly by GFR and is not affected by sex, or by inflammation or changes in muscle mass (Abrahamson et al. 1990; Grubb 1992). However, Knight et al. (2004) observed higher serum CRP to be associated with higher serum cystatin C after adjusting for creatinine clearance. Nonetheless, inflammation was not confirmed to be an interfering factor in the present study. There have also been reports of interference of RF with cystatin C measurement (Lamb and Stowe 2003). The non-specific agglutination caused by RF could increase the apparent concentration of cystatin C. Such a finding was not confirmed in a fore-mentioned study by Kyhse-Andersen et.al. (1994), nor in the present study. The interference has been suggested to be related to the different reagents or to varying methods used in the determination of cystatin C (Newman 2003). In the present study, similarly to that by Kyhse-Andersen (1994), an immunoturbidimetric assay was applied, whereas in the study finding non-specific agglutination (Lamb and Stowe 2003), a nephelometric assay was used.

There are thus contradictory reports on the influence of inflammation and RF on the levels of cystatin C, but results on the effect of glucocorticoids on cystatin C levels have been more congruent. Glucocorticoids may increase cystatin C values, leading to systematic underestimation of GFR (Cimerman et al. 2000; Risch et al. 2001; Wasen et al. 2003). The effect is transitory and has been
estimated to be 0.20-1.85 mg/l in patients receiving low-dose glucocorticoids and even higher in those receiving high-dose glucocorticoids (Risch et al. 2001). In the present study almost all patients received low-dose glucocorticoids and the influence of glucocorticoid dosing could not be evaluated.

Serum or plasma creatinine is widely considered an unreliable marker of renal function and also in the present study its performance was at best moderate. It had the lowest correlation with $^{51}$Cr-EDTA clearance and it left 42% of GFR < 90 ml/min/1.73m$^2$ undetected, while the percentage was 12 when the GFR was estimated by the CG formula. Reports on the poor reliability of serum creatinine concentration as an index of GFR in RA patients have previously been published (Nived et al. 1983; Laiho et al. 2001), but in only one study was the GFR measured directly (Nived et al. 1983). In this latter study, the control patients had higher serum creatinine levels than the patients with rheumatic diseases, even when the levels of directly measured GFR were equal in both groups. Estimation of GFR by serum creatinine or creatinine clearance is affected by muscle mass changes, diet, physical activity and inflammatory processes (Perrone et al. 1992). Due to inactivity and inflammation, muscle mass may be reduced in RA patients, resulting in lower creatinine production (Nived et al. 1983; Boers et al. 1988).

Measurement of plasma urea offered no noteworthy benefits compared to measurement of plasma creatinine. It identified slightly better patients with decreased GFR than plasma creatinine, but the correlation between $^{51}$Cr-EDTA clearance and plasma urea was no better than that between $^{51}$Cr-EDTA and plasma creatinine. Furthermore, plasma urea is held to be unsuitable as a single measure of renal function in that it is influenced by variations in urine flow rate as well as in the production and metabolism of urea (Stevens and Levey 2005).

In conclusion, creatinine clearance showed the best correlation with GFR, but the correlations of the CG and MDRD formulas were almost as good. Plasma cystatin C was better than plasma creatinine measurement, but still too insensitive to identify GFR < 90ml/min/1.73m$^2$. Use of the
creatinine-based formula or creatinine clearance measurement for estimation of RA patients’ GFR can be recommended instead of using solely plasma creatinine or cystatin C measurement.

5. Limitations of the study and future directions

There were some methodological problems in follow-up studies I and II. Probably the most prominent limitation was that the analysis in study I and also in II had to be based on hospital records for a notable proportion of patients. Half of the original patients in study I were deceased and some were not able to attend the follow-up appointment by reason of poor physical condition. One source of bias could be the younger age of patients participating in study I compared to those studied by hospital records. In the RA patients studied from records, analysis of urinary findings had to be based mostly on urine dipstick findings. The weakness relating to single dipstick findings was realized, but the high death rate among the study patients and the advanced age of those remaining made it impossible to carry out the study in any other way. On the other hand, only few patients had moved to other districts. The hospital records were quite readily available in the two hospitals in the district, and notes in patient records were usually highly informative, increasing the reliability of results. A further strength of the study was the long follow-up time, the median being 13 years. Finally, the RA control group in study I was selected given the study settings in 1988, and this must be kept in mind in interpretation of results concerning RA controls.

Study III was the first study ever to correlate plasma cystatin C to an accurate indicator of GFR based on measurement of exogenous substances ($^{51}$Cr-EDTA) in RA patients, and it was also the first
to compare six currently most used measures of renal function in the same RA patient population. Increasing the number of patients in this study could still have given more information on possible interfering factors concerning the measurement of cystatin C. Study III emphasizes the value for a clinician of awareness of the GFR in RA patients. For example methotrexate, which is currently one of the DMARDs most widely used in clinical practice, is secreted by the kidneys and its dosage should be adjusted in patients with GFRs < 60 ml/min/1.73m$^2$. Nonetheless, according to one recent paper (Karie et al. 2008) even half of the RA patients studied did not have appropriate methotrexate dose adjustment according to their stage of kidney function. The serum creatinine level does not exceed the range of normal until as much as 50 per cent of total GFR is lost (Brenner et. al. 1987). In patients with RA the sensitivity of serum creatinine measurement is even lower by reason of muscle mass reduction (Herbison et al. 1987; Miro et al. 1996). Study III clearly demonstrates that serum creatinine or cystatin C measurements are not adequate for the estimation of GFR in RA patients and rheumatologists and other clinicians should use CG formula or alternatively creatinine clearance more widely in clinical practise.

The limitation in study IV was that the estimations of the occurrence of proteinuria and hematuria were based on dipstick analyses of urine. However, multiple samples from single patients were available during the long follow-up and the main result was based on the occurrence of repeated findings (≥3 times) to increase the reliability of conclusions. Secondly, detailed information on the use of single DMARDs was not analyzed, but the association of the usage of single nephrotoxic DMARDs (DPA, gold salts, and cyclosporine) with cases of proteinuria and impaired renal function was defined. Study IV showed that nephrological side-effects are not a concern in the FIN-RACo combination DMARD strategy including sulfasalazine, methotrexate, hydroxychloroquine and low-dose prednisolone. However, the finding does not obviate the need for regular monitoring for changes in urinalysis and kidney function in RA patients in general.
The treatment of RA has essentially become more effective during the past decade and remission is nowadays a realistic target for treatment. There is a shortage of new prospective data on the incidence of AA-amyloidosis in patients with RA. In the future it will be important to evaluate the occurrence of AA-amyloidosis during the modern DMARD treatments and biologicals, and also the latency in the appearance of clinical manifestations after the first evidence of AA-amyloidosis, e.g. a positive subcutaneous biopsy result.

6. Clinical implications of the study

In the case of RA patients’ hematuria the urological cause of the finding should be primarily excluded. Since no clinical association has been established between isolated microhematuria and use of DMARD (White et al. 1984; Leonard et al. 1987; Korpela et al. 1995; Koseki et al. 2001) or NSAID therapies (Richards et al. 1988; Korpela et al. 1995; Koseki et al. 2001), there is no basis for discontinuation of this therapy. The most frequent renal histological diagnosis related to microhematuria is MesGN, and the favorable long-term prognosis of the condition was here confirmed. Furthermore, isolated hematuria, even glomerular, is not usually an indication for renal biopsy at present, and after urological investigations the finding can be followed up at longish intervals.

In the case of proteinuria a positive screening test must first be confirmed by diurnal urine collection. Protein excretion exceeding 500 mg/day warrants the discontinuation of nephrotoxic DMARDs (gold salts and DPA) and avoidance of NSAIDs. If proteinuria continues for several months, showing no response to cessation of nephrotoxic DMARDs, referral to a nephrologist and renal biopsy is to be considered. The possibility of AA-amyloidosis should be borne in mind, especially in advanced
RA patients with prolonged high disease activity, proteinuria and/or renal insufficiency. To retard the progression of AA-amyloidosis the underlying inflammation should be controlled with intensified anti-rheumatic treatment (Ahlmen et al. 1987; Berglund et al. 1993; Elkayam et al. 2002).

On the basis of the present findings, the GFR of RA patients is recommended to be estimated using the CG formula or creatinine clearance. The sensitivities of serum creatinine or cystatin C measurements are not satisfactory in patients with RA. It is of vital importance to adjust the dosage of medications excreted by the kidneys (e.g. methotrexate, hydroxychloroquine) and avoid NSAIDs and nephrotoxic DMARDs such as cyclosporine, gold salts and DPA in RA patients with estimated GFR <60ml/min/1.73m$^2$. 
SUMMARY AND CONCLUSIONS

1. In patients with long-lasting RA and clinical nephropathy findings in the cross-sectional population-based study in 1988, isolated proteinuria and proteinuria combined with hematuria and/or CRF very frequently continued over the 13-year follow-up. RA patients with these findings had poor clinical long-term prognosis, mostly by reason of underlying renal amyloidosis. Isolated hematuria and isolated CRF were associated with evidently better prognosis with good preservation of renal function and infrequent need for dialysis therapy. New abnormal renal findings were recorded in almost one third of RA patients, but these findings were mostly mild in character.

2. Mesangial glomerulonephritis had a favorable clinical prognosis in the 15-year follow-up, whereas renal AA-amyloidosis was associated with evidently poorer prognosis with frequent appearance of serious impairment of renal function and frequent need for dialysis therapy.

3. Creatinine clearance and the CG formula had the best diagnostic ability to identify RA patients with reduced GFR. Sole use of the measurement of plasma creatinine, cystatin C or urea to estimate the GRF of RA patients cannot be recommended in clinical practice.

4. The cumulative incidence of repeated hematuria findings in the 11-year follow-up of early RA patients was high, the incidence of repeated proteinuria and raised serum creatinine being evidently more infrequent. The repeated finding of eGFR_{CG}<60\text{ml/min}/1.73\text{m}^2 was made in
over 10% of patients. Single nephrotoxic DMARDs were in only few cases associated with the occurrence of proteinuria or CRF. The initial remission-targeted therapy with the FIN-RACo combination DMARD therapy in early RA was safe for the kidneys and induced no more short- or long-term renal complications compared to therapy with a single DMARD.
ACKNOWLEDGEMENTS

This study was carried out at the Department of Internal Medicine, Tampere University Hospital, and at the Medical School, University of Tampere, Finland.

I am truly grateful to my supervisors Professor Jukka Mustonen, M.D. and Docent Markku Korpela, M.D. for their patience and support during this work. They introduced me to the scientific field of rheumatology and nephrology and their help and guidance were prerequisite to the completion of this study.

I wish to express my warmest gratitude to Docent Heikki Saha, M.D. for his contribution to the planning and implementation of the study of measures of renal function. His patient guidance and encouraging attitude towards the whole undertaking has been invaluable.

I wish express my sincere thanks to my co-authors Professor Pekka Hannonen, Docent Aimo Harmoinen, PhD, Professor Marjatta Leirisalo-Repo, Professor Timo Möttönen, and Vappu Rantalaiho, M.D. for their invaluable contribution to the study. I am greatly indebted to Docent Heikki Helin, M.D. for the histological diagnostics and Hannu Kautiainen, B.A. and Professor Terho Lehtimäki, M.D. for their invaluable part in the statistical analysis of my findings. I also warmly thank all the members of the FIN-RACo Trial group for their tireless work with the trial and also for letting me utilize their accomplishments in my study.

I am deeply indebted to my co-author and dear colleague Susanna Sihvonen, M.D. for her significant contribution to the study, for her heartening words in my melancholy moments, and for her endless optimism over the outcome. I also owe thanks to my close workmate nurse Heidi Marika Mäkinen for her invaluable assistance with the study patients and materials and for her always so encouraging attitude towards the study. Her professional skills and humane sensitivity have brought pleasure to my everyday work during the past years. I extend my warmest thanks to the entire personnel of the Department of Rheumatology for encouraging me in my efforts.

I warmly thank Docent Kari Pietilä, M.D. and Docent Jaakko Antonen, M.D. Heads of the Department of Internal Medicine, for providing me the facilities to carry out this study and for a positive and understanding attitude towards my study.
I sincerely thank the official reviewers, Docent Agneta Ekstrand, M.D. and Docent Jorma Viitanen, M.D., for their valuable comments and constructive criticism, which greatly improved the quality of this thesis.

I am most thankful to Mr Robert Mc Gilleon, MA for careful and swift revision of the English language of this thesis.

My warmest thanks belong to my fellow-student Johanna Palmio for our weekly squash matches and our lively conversations about everything from the academic issues to gardening during breaks. Those moments greatly helped me to maintain a proper perspective on everyday life during these years. I am also grateful to Sinikka Forsberg, M.D. for introducing me to the interesting world of rheumatology, setting a good example of clinical expertise and human approach.

I warmly thank my parents Mervi and Keijo Vidqvist for their never-ending support and encouragement and being so proud of everything I have done.

I dedicate this book to my family, who have supported me throughout these years. To my older daughter Eerika, whose growth into a young lady I have proudly followed, and to my younger daughter Annika, whose sparkling girl-power brings laughter to my life. And finally to my beloved husband Tuomas, the light of my life, who has unselfishly made it possible for me to concentrate on this work. Life with him has been filled with love, companionship and new adventures and I am privileged to have shared all these years with him.

This work was financially supported by grants from the Competitive Research Funding of the Pirkanmaa Hospital District, the Finnish Kidney Foundation, the Scandinavian Rheumatology Research Foundation, Tampereen Reumayhdistys, the Local Branch of the Finnish Rheumatism Association, and the Finnish Society for Rheumatology.

Tampere, March 2009

Krista Karstila
REFERENCES:


II. Reprinted with permission from Dustri-Verlag Dr Karl Feistle GmbH & Co. KG: *Clinical Nephrology*; 68: 335-336 © 2007


Measurement of the kidney function in patients with rheumatoid arthritis: plasma cystatin C versus $^{51}$Cr-EDTA clearance

Krista Karstila$^a$, Aimo P.T. Harmoinen$^b$, Terho J. Lehtimäki$^{c,d}$, Markku M. Korpela$^a$, Jukka T. Mustonen$^{a,d}$, Heikki H.T. Saha$^{a,d}$

$^a$Department of Internal Medicine, Tampere University Hospital, Tampere

$^b$Department of Clinical Chemistry, Savonlinna Central Hospital, Savonlinna,

$^c$Laboratory of Atherosclerosis Genetics, Department of Clinical Chemistry, Center for Laboratory Medicine, Tampere University Hospital, and $^d$Medical School, University of Tampere, Tampere, Finland

This study was conducted at the Department of Internal Medicine, Tampere University Hospital.

Key Words: Chronic renal disease, rheumatoid arthritis, Creatinine clearance, Cystatin C, Glomerular filtration rate, Rheumatoid arthritis, kidney function

Address correspondence and reprint requests to Krista Karstila MD, Department of Internal Medicine, Tampere University Hospital, P.O. Box 2000, Fin-33521, Tampere, Finland. Tel: + 358-3-311 63473, Fax +358-3-311 64362, E-Mail: krista.karstila@ fimnet.fi
Abstract

Background/Aim: Knowledge of the usefulness of cystatin C measurement in the detection of chronic kidney disease in patients with rheumatoid arthritis (RA) is scant. The purpose of this study was to evaluate the ability of plasma cystatin C- and creatinine-based methods to predict glomerular filtration rate (GFR) and classify chronic kidney disease in RA patients.

Methods: The study population consisted of 64 RA patients aged 41-86 years. Comparisons were made between measured plasma creatinine, cystatin C, creatinine clearance and GFR estimated by the Cockcroft-Gault (GC) and the Modification of Diet in Renal Disease (MDRD) formulas. The plasma clearance of $^{51}$Cr-EDTA served as a reference.

Results: The Pearson correlation coefficients between plasma clearance of $^{51}$Cr-EDTA and the markers of GFR were calculated. The correlation coefficients were 0.800 for plasma creatinine, 0.863 for cystatin C, 0.866 and 0.904 for GFR values estimated by MDRD and CG and 0.922 for plasma creatinine clearance. Statistically significant differences were detected between the correlation coefficients of plasma creatinine and GFR estimated by CG (p=0.0412) and plasma creatinine and creatinine clearance (p=0.0099). Creatinine clearance and the MDRD and CG formulas proved to be better at identifying GFR< 90ml/min than plasma creatinine or cystatin C.

Conclusion: We recommend using the CG formula or creatinine clearance for the estimation of the GFR of RA patients instead of solely creatinine or cystatin C in clinical work.
Introduction

Impairment of kidney function (reduced glomerular filtration rate, GFR) is common in patients with rheumatoid arthritis (RA) [1,2]. Both drugs and complications of RA have been implicated in the etiology of kidney disease [2-6].

In RA patients it is essential to estimate the GFR precisely to optimise the choice and dosage of medical treatment, and to avoid further renal damage or adverse events. Methods based on the measurement of exogenous substances (e.g. inulin, $^{51}$Cr-EDTA) are accurate indicators of GFR, but are too laborious and expensive to be used in clinical practice. Plasma or serum creatinine and its endogenous renal clearance are the most widely used methods, but in RA patients a great number of interfering factors such as reduced muscle mass, immobilization and inflammation accompany the use of these indicators [7]. To overcome the problems associated with plasma creatinine and creatinine clearance measurements, more than 25 different [8] formulas have been developed to estimate GFR from prediction equations.

Cystatin C is a 13-kDa protein produced at a constant rate by all nucleated cells [9]. The serum and plasma concentrations of cystatin C are mainly determined by GFR and are unaffected by changes in muscle mass, sex or inflammatory stage [10, 11], although opposite has also been stated [12]. Knowledge of its usefulness in the detection of renal function in RA patients is scant.

To establish which is the most accurate means of assessing GFR in RA patients, we compared measurements of plasma cystatin C, creatinine, creatinine clearance and creatinine-based formulas against plasma $^{51}$Cr-EDTA clearance. The data on the correlations of these measurements with accurate direct measurements of GFR in RA patients are limited [1, 13, 14]. Cystatin C and Modification of Diet in Renal Disease (MDRD) formula have been validated in large patient
cohorts, but as far as we know, this is the first study in RA patients to correlate plasma cystatin C and MDRD formula with an accurate indicator of GFR ($^{51}$Cr-EDTA) instead of using creatinine clearance as a reference method.

**Patients and methods**

*Patients*

Sixty-four patients (47 women and 17 men) with RA were included in the study. Patients with diverse body composition and assumed renal function were enrolled and all fulfilled the American Rheumatology Association 1987 criteria for RA [15]. Rheumatoid factor (RF) was positive in 44 of them. The age of the patients was $66 \pm (SD) 11$ years (range 41- 86 years) and the duration of RA $21 \pm (SD) 13$ years (range 0-49). The body mass index was $25\pm5$ (range 15-34). The glucocorticoid dosing was $\leq 10$ mg prednisolone in 51 patients and 15-20 mg in 7 patients, while 6 patients were not on corticosteroid therapy. Two of the patients were on medication that could inhibit the secretion of creatinine. Both of them were using trimethoprim and the daily dose was 50 mg for the one and 100 mg for the other.

The study was carried out in compliance with the Helsinki Declaration and approval of the study was obtained from the Ethical Committee of Tampere University Hospital. After written informed consent had been obtained, laboratory determinations were performed in conjunction with a routine follow-up appointment in the outpatient ward of the Department of Nephrology or Rheumatology.
Methods

Plasma $^{51}$Cr-EDTA clearance was assessed by single injection method and blood samples drawn after 0, 90 and 180 min [16]. A part of 0-fasting sample was used to determine plasma creatinine and cystatin C. Also 24-hour timed urine collections were obtained to determine endogenous creatinine clearance related to 1.73 m² of body surface area. Endogenous creatinine clearance (ml/min) was calculated as follows:

$$\frac{V(\text{ml})}{1440\text{min}} \times \left(\frac{\text{urine creatinine (}\mu\text{mol/l}) \times 1.73/\text{A}}{\text{plasma creatinine (}\mu\text{mol/l})}\right)$$

where $V=$ the 24-hour volume of urine (ml), and $A=$ body surface area (m²)

Written instructions for fasting and urine collections were given by trained nurses. Patients were advised to start collecting urine in the morning between 6 and 7 a.m. Before the collection was started, the patients were advised not to include the first urine sample. All the urine passed during the next 24 h was collected into a standard plastic container and stored at 2-8°C and any loss of urine however small led to the rejection of the whole collection. Patients were also advised to live as normally as possible during the urine collection; limitations in diet or physical activity were discouraged.

Plasma cystatin C was measured immunoturbidimetrically on a Hitachi 704 analyser or on a Cobas Mira and Integra instrument (F.Hoffman-La Roche Ltd., Basel, Switzerland). Plasma creatinine was determined enzymatically using the same instruments [17]. The intra- and interassay coefficients of variation of plasma creatinine were 1.7 and 1.9%, respectively. Serum RF and C-reactive protein (CRP) were determined immunoturbidimetrically on the same instruments. The erythrocyte sedimentation rate (ESR) was measured automatically by a Monitor V100 AUTO e.s.r. Analyser (Electa Lab, Forli, Italy).
The reference values for plasma creatinine were < 95 µmol/l for women and < 105 µmol/l for men. The reference values for plasma cystatin C were < 1.2 mg/l for individuals ≤ 50 years of age and < 1.4 mg/l for those aged > 50 years.

In addition, the GFR was estimated according to a modified Cockcroft-Gault (CG) [18] formula:

$$\text{eGFR/CC} = \frac{((140-\text{age}) \times \text{body weight (kg)})}{(\text{plasma creatinine (µmol/l)} \times a)}$$

where a = 0.8 if male, and 0.95 if female, and the MDRD [19] formula:

$$\text{eGFR/MDRD} = 186 \times (\text{plasma creatinine (µmol/l)/88.4})^{1.154} \times \text{age}^{-0.203} \times (0.742 \text{ if female}).$$

When studying the possible influence of RF on plasma cystatin C values, the plasma cystatin C values were expressed as milligrams per liter first converted to GFR (ml/min) using the following formula [20]:

$$\text{eGFR/CystC} = 94.652 \times \text{plasma cystatin C}^{-1.2478}$$

After that, eGFR/CystC was reduced by the GFR measured by plasma $^{51}$Cr-EDTA clearance and the difference correlated to the concentration of RF in the sample.

**Statistical analysis**

Comparisons were made between GFR as assessed by the plasma clearance of $^{51}$Cr EDTA and the levels of plasma cystatin C, creatinine, creatinine clearance and estimated GFR using the CG and MDRD formulas. Comparisons between plasma cystatin C and creatinine were made using reciprocals of the concentrations, since the plasma concentration of these substances is inversely related to their clearance. The Pearson correlation coefficient was used for correlation analysis. The comparison of the differences between correlation coefficients was made using the Statistica for Windows version 6.0 (StatSoft, Inc., Tulsa, Okla., USA). The differences between the sensitivities
of the tests to identify patients with a reduced GFR (<90 ml/min/1.73m$^2$) were analysed by $\chi^2$ or Fisher’s exact test. Differences between plasma $^{51}$Cr-EDTA clearance and the CG estimates for GFR and plasma $^{51}$Cr-EDTA clearance and creatinine clearance were also studied by using Bland-Altman plots [21]. The partial correlation test was used after adjusting for RF, CRP and ESR to study the possible effect of these variables on correlation coefficients between cystatin C and $^{51}$Cr-EDTA. For all tests, $p$-value < 0.05 was considered significant.

**Results**

All the parameters studied to estimate the severity of chronic kidney disease (CKD) are shown in table 1. The kidney function studied by $^{51}$Cr-EDTA clearance was categorized according to the Kidney Disease Outcome Quality Initiative (K/DOQI) guidelines of the National Kidney Foundation of the USA [8]. Kidney function was normal, stage 1 (GFR ≥ 90 ml/min/1.73m$^2$) in 4 patients, mildly decreased, stage 2 (GFR=60-89 ml/min/1.73m$^2$) in 17, moderately decreased, stage 3 (GFR=30-59 ml/min/1.73m$^2$) in 25 and severely decreased, stage 4 (GFR=15-29 ml/min/1.73m$^2$) in 18. None of the patients had kidney failure, stage 5 (GFR<15 ml/min/1.73m$^2$). CRP was available for 42 patients and ESR for 38 patients. The median of CRP was 8.8 mg/l (range 1.2-51.6 mg/l) and of ESR 25 mm/h (range 2-74).

The sensitivity of the tests to identify patients with reduced GFR is shown in table 2. When the plasma $^{51}$Cr-EDTA clearance falls below 90 and 60 ml/min/1.73m$^2$, in 42% and 19% of the patients, respectively, the plasma creatinine still remains within the normal range. The plasma cystatin C remained within the normal range in 29 and 12% of the patients with stage 2 (GFR<90ml/min) or stage 3 CKD (GFR<60ml/min), respectively, while the CG and MDRD estimates for GFR and plasma creatinine clearance showed essentially better sensivity.
Creatinine clearance (p< 0.0001, $\chi^2$–test), cystatin C (p< 0.0001, $\chi^2$–test) and the MDRD (p=0.004, $\chi^2$–test) and CG (p=0.017, $\chi^2$–test) formulas were better at identifying patients with decreased GFR (< 90ml/min) than plasma creatinine. Also creatinine clearance (p=0.001, $\chi^2$–test) and the MDRD and CG formulas (both p=0.007, $\chi^2$–test) proved to be better at identifying patients with decreased GFR than plasma cystatin C.

Correlations of the measured parameters with $^{51}$Cr-EDTA are shown in Figure 1. The correlation coefficient between plasma creatinine and $^{51}$Cr-EDTA clearance (r=0.800) was lower than seen between plasma $^{51}$Cr-EDTA clearance and GFRs estimated by plasma cystatin C (r=0.863), MDRD (r=0.866) and CG formulas (r=0.904) and creatinine clearance (0.922). However, a statistical difference in correlation coefficients was seen only between plasma creatinine and CG (p= 0.0412) and between plasma creatinine and creatinine clearance (p=0.0099). Partial correlation coefficients between plasma cystatin C and $^{51}$Cr-EDTA clearance after adjusting for RF, CRP or ESR did not differ significantly from the nonadjusted correlation coefficients. The level of RF had no influence on plasma cystatin C levels either.

The level of the GFR estimated by the GC and MDRD formulas appeared to be approximately 20 % higher than measured with plasma $^{51}$Cr-EDTA clearance. Figure 2 shows that the dispersion of the CG estimates increased at GFRs > 60 ml/min. Also creatinine clearance seemed to be more inaccurate in patients with GFRs > 60ml/min (Figure 2).
Discussion

Our study is the first to correlate plasma cystatin C with an accurate indicator of GFR based on measurement of exogenous substances ($^{51}$Cr-EDTA) in RA patients. Mangge et al. [22] showed that plasma cystatin C is better than plasma creatinine in detecting early renal impairment in patients with prolonged RA (n=56), but creatinine clearance was used as reference method.

In RA patient many factors (glucocorticoids, rheumatoid factor, inflammation) may have an effect on plasma cystatin C [12,23-27]. Glucocorticoids may increase cystatin C values, leading to systematic underestimation of the GFR [23-25]. The effect is transitory and dose dependent [24]. In the present study, almost all patients received low dose glucocorticoids and the influence of glucocorticoid dosing could not be evaluated. RF can bind to the Fc region of the immunoglobulin G molecules and cause nonspecific agglutination [26]. This could increase the apparent concentration of cystatin C. We found no correlation between the concentrations of RF and cystatin C, which is in accord with the report of Kyhse-Andersen et al. [27]. There are contradictory reports on the effect of inflammatory stage on plasma cystatin C [10-12]. In the present study, we could not find any influence of inflammatory parameters (CRP, ESR) on cystatin C values.

The concentration of serum or plasma creatinine is the most widely used measure of kidney function in RA patients in clinical practice, though it clearly overestimates GFR [7]. In our study measurement of plasma creatinine left 42% of stage 2 CKD undetected, while the percentage was 12 when GFR was estimated by the GC formula. There have been earlier reports on the poor reliability of the creatinine concentration as an index of GFR in RA patients [13, 28], but the study populations in these studies have been relatively small and only in one study was the GFR measured directly [13]. In that study Nived et al. [13] showed in 34 RA and 20 spondylarthritis patients that patients with rheumatic diseases had lower serum creatinine levels than control patients.
with an equal GFR. Estimation of GFR by creatinine or creatinine clearance may be affected by muscle mass changes, diet, physical activity and inflammatory processes [7]. Due to inactivity and inflammation, the muscle mass may be reduced in RA patients, resulting in a lower creatinine production [13, 29]. The sensitivity of creatinine in indicating impairment of kidney function is particularly low in muscle-wasted individuals [30]. Our results clearly show that plasma creatinine measurement is too insensitive to estimate the GFR in RA patients.

Endogenous creatinine clearance has been considered a more sensitive marker of GFR than serum creatinine, but direct comparisons between creatinine and creatinine clearance measurements correlated with direct measurements of GFR have not been done in RA patients. Perhaps slightly surprisingly, creatinine clearance proved to be an indicator of GFR at least as good as creatinine-based GFR estimates in RA patients. It requires 24-hour collection of urine, making the measurement laborious and slow. Creatinine clearance is also prone to collection inaccuracies and physiological and analytical problems [7] and there have even been calls for its abandonment by laboratories and clinicians [31]. The good functioning of the creatinine clearance in our RA population may be related to the long experience and good training of our patients in this method.

The GC formula [18] was found to be a better indicator of mild (stage 2) CKD than plasma creatinine or cystatin C. The CG formula has originally been developed to predict creatinine clearance, but is nowadays widely used as an estimate of the GFR in the literature. The correlation coefficient of GC prediction with the measured creatinine clearance has been high in RA patients (0.91) [29] and slightly lower (0.82) with the direct measurement of GFR [14]. Our results were comparable with these former reports.
The CG formula has been developed using alkaline picrate methods, which independent of the level of plasma creatinine overestimate plasma creatinine by an average of 12 µmol/l (0.14mg/dl) [32]. This leads to higher estimated GFR levels when the plasma creatinine used in the formula is determined enzymatically, as was also found in our study. Our result agrees with earlier reports finding the CG formula an adequate tool to detect GFR impairment in RA patients [14, 29]. Altogether, in the present study, CG functioned as well as creatinine clearance and it is far easier to use in clinical practice, requiring only the measurement of plasma or serum creatinine.

The divergence in the CG and MDRD equations for the estimation of GFR values increases at stage 1 and 2 CKD and it has even been recommended not to report numerical values for an estimated GFR >60 ml/min/1.73m² [33]. In subjects with a normal or increased GFR, the MDRD formula has been reported to be less accurate than creatinine clearance or the CG formula [34]. In RA patients the CG formula has shown a better correlation with creatinine clearance than the MDRD formula (r= 0.69 vs 0.41) [35]. In the present study, this formula showed a slightly weaker correlation with GFR assessed using $^{51}$Cr-EDTA than did the CG formula. Further, the MDRD has been shown to overestimate GFR for the same reason as the CG formula [33].

**Conclusions**

It is of vital importance for a clinician to be aware of the GFR of RA patients. When the GFR falls below 60ml/min, it has an essential influence on the selection and dosage of the medication. Cystatin C was better than plasma creatinine measurement, but still too insensitive to identify stage 2-3 CKD. Creatinine clearance showed the best correlation with GFR, but the CG formula was not significantly inferior. We recommend the use of the GC formula or creatinine clearance measurement for estimating the GFR of RA patients instead of using solely creatinine or cystatin C measurement.
Acknowledgements

This study was financially supported by the competitive research funding of the Pirkanmaa Hospital District and Tampereen Reumayhdistys, the Local Branch of the Finnish Rheumatism Association.
References


Table 1. Parameters representing the kidney function

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma $^{51}$CrEDTA, ml/min/1.73m$^2$</td>
<td>44</td>
<td>17-135</td>
</tr>
<tr>
<td>Plasma cystatin C, mg/l</td>
<td>1.57</td>
<td>0.62-4.55</td>
</tr>
<tr>
<td>Plasma creatinine, μmol/l</td>
<td>104</td>
<td>39-475</td>
</tr>
<tr>
<td>Creatinine clearance, ml/min/1.73m$^2$</td>
<td>47</td>
<td>8-145</td>
</tr>
<tr>
<td>Estimated GFR/MDRD, ml/min/1.73m$^2$</td>
<td>54</td>
<td>6-149</td>
</tr>
<tr>
<td>Estimated GFR/CG, ml/min/1.73m$^2$</td>
<td>53</td>
<td>9-156</td>
</tr>
</tbody>
</table>
Table 2. Proportions of the various markers of GFR showing incorrectly normal values even when the kidney function is mildly or moderately impaired ($^{51}$Cr-EDTA clearance <90 or <60 ml/min/1.73m$^2$)

<table>
<thead>
<tr>
<th>Test</th>
<th>$^{51}$Cr-EDTA &lt; 90 ml/min/1.73m$^2$</th>
<th>$^{51}$Cr-EDTA &lt; 60 ml/min/1.73m$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma creatinine (n=64)$^a$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal/all</td>
<td>25/60 (42%)</td>
<td>8/43 (19%)</td>
</tr>
<tr>
<td>Plasma cystatin C (n=62)$^b$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal/all</td>
<td>17/58 (29%)</td>
<td>5/42 (12%)</td>
</tr>
<tr>
<td>GFR/MDRD (n=64)$^c$, ≥90 ml/min/all</td>
<td>6/60 (10%)</td>
<td>0</td>
</tr>
<tr>
<td>GFR/CG (n=64)$^d$, ≥90 ml/min/all</td>
<td>7/60 (12%)</td>
<td>1/43 (2%)</td>
</tr>
<tr>
<td>Creatinine clearance (n=54) ≥90 ml/min/all</td>
<td>8/50 (16%)</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$ Differentiation limit: 95 µmol/l for women, 105 µmol/l for men.

$^b$ Differentiation limit: 1.2 mg/ml for individuals ≤50 years, 1.4 mg/ml for those > 50 years

$^c$ GFR estimated by MDRD formula, differentiation limit: 90 ml/min/1.73m$^2$.

$^d$ GFR estimated by CG formula, differentiation limit: 90 ml/min/1.73m$^2$. 
Fig. 1. Correlations between GFR assessed using $^{51}$Cr-EDTA clearance and 100/plasma creatinine (a), 1/plasma cystatin C (b), MDRD (c) and CG (d) equations for estimation of GFR, and creatinine clearance (CrCl, e) in patients with RA.

a) $y = 0.0153x + 0.2362$
   $r = 0.800, n=64$

b) $y = 0.091x + 0.1827$
   $r = 0.863, n=62$

c) $y = 1.1067x + 2.6449$
   $r = 0.866, n=64$

d) $y = 1.2202x - 2.2127$
   $r = 0.904, n=64$

e) $y = 1.198x - 3.3003$
   $r = 0.922, n=54$
Fig. 2. Difference between $^{51}$Cr-EDTA clearance and the CG estimates for GFR (a) and creatinine clearance (CrCl) (b) studied by Bland–Altman plots.