The chemokine receptor 5 Δ32 mutation is associated with increased renal survival in patients with IgA nephropathy

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The chemokine receptor 5 Δ32 mutation is associated with increased renal survival in patients with IgA nephropathy.

Background. Chemokine receptor 5 (CCR5) plays an important role in the recruitment of monocytes and T cells in inflammation and experimental studies suggest that CCR5 might be involved in the pathogenesis of IgA nephropathy. A mutation in the CCR5 gene (CCR5 Δ32), leading to a nonfunctional receptor, was recently described. We therefore evaluated the potential role of this mutation on renal survival in patients with IgA nephropathy.

Methods. The distribution of the CCR5 Δ32 genotype was determined by polymerase chain reaction (PCR) analysis in 228 patients with biopsy-proven IgA nephropathy. In 190 patients with available demographic and clinical follow-up data, the effect of the mutation on the clinical outcome was analyzed using the Log-rank test and the Cox proportional hazard model. In vitro, the influence of the CCR5 Δ32 genotype on the chemotactic response of monocytes was assessed.

Results. Of the 190 patients, 158 (83.2%) had a CCR5 wild-type genotype, 29 (15.3%) were heterozygous, and three patients had a homozygous CCR5 Δ32 genotype (1.6%). Renal survival was significantly longer in patients with the CCR5 Δ32 genotype than in the wild-type group (Log-rank P < 0.001). Using the multivariate Cox proportional hazard model, the CCR5 Δ32 genotype was identified as an independent factor associated with a lower risk to develop end-stage renal disease (ESRD) [hazard ratio (HR) 0.23, 95% CI 0.09 to 0.57, P = 0.002]. In vitro analysis of monocytes from CCR5 Δ32 carriers showed a reduced chemotactic response to CCR5 ligands in vitro.

Conclusion. Our study demonstrates an independent role of the CCR5 Δ32 genotype for the clinical outcome in IgA nephropathy. In vitro experiments revealed a reduced chemotactic response of monocytes from CCR5 Δ32 carriers, thus pointing out a possible pathophysiologic explanation for the beneficial effect of the CCR5 Δ32 genotype.

The infiltration of inflammatory leukocytes into the glomerulus and the renal tubulointerstitium are hallmarks of most forms of human glomerulonephritis [1, 2]. Inflammatory cell infiltrates mediate injury and repair. This is also true in patients with IgA nephropathy, the most frequent form of glomerulonephritis in the world. The clinical course of IgA nephropathy is characterized by stable renal function in the majority of patients. One third of the patients, however, loses renal function and progresses to end-stage renal disease (ESRD) [3–5]. Although the mechanisms responsible for disease progression are largely unknown a correlation between the presence of mononuclear inflammatory cells (monocytes and lymphocytes) in renal tissue and the progression of the disease exists [6–8].

Chemokines, a group of chemotactic cytokines, play a central role in the recruitment of leukocytes [9]. Their effects are mediated through chemokine receptors, which are predominantly expressed on leukocytes [9, 10]. An important receptor on monocytes and T cells is the chemokine receptor 5 (CCR5). Experimental data in animals show that blockade of the CCR5 receptor with a peptide antagonist reduces glomerular leukocyte infiltration and ameliorates the development of renal injury [11]. Importantly, the number of infiltrating CCR5-positive leukocytes into the kidney is positively correlated with loss of renal function in IgA nephropathy, suggesting a role for CCR5 in the pathophysiology of this glomerulonephritis [12].

Recently, a 32 bp deletion mutation in the CCR5 gene (CCR5 Δ32) was described which leads to a nonfunctional receptor. The CCR5 Δ32 allele frequency is between 0.04 and 0.16 of humans in Europeans [13]. The
high allelic frequency of CCR5 Δ32 in the European and North American population cannot be explained through random genetic drift, suggesting an unknown selection advantage of allele carriers [13]. Homozygous carriers appear to be healthy and are protected against human immunodeficiency virus (HIV) infection since the CCR5 is a coreceptor for the M tropic variant of HIV [14–16]. Other, preliminary reports demonstrated a reduced CCR5 Δ32 allele frequency in patients with asthma [17]. In rheumatoid arthritis, disease severity was reduced in patients carrying the CCR5 Δ32 genotype [18]. A prolongation of renal transplant survival was found in patients with the homozygous form of the CCR5 Δ32 mutation [19]. These data suggest an important role of the CCR5 in diseases characterized by the recruitment of mononuclear cells.

The aim of our study was to evaluate the effect of the CCR5 Δ32 genotype on the clinical course of patients with IgA nephropathy. A total of 190 patients from German Nephrology centers with biopsy-proven IgA nephropathy and clinical follow-up data were included in the study and the effect of the mutation on renal survival was analyzed. In an attempt to determine possible pathophysiologic differences we assessed the influence of the CCR5 Δ32 genotype on monocyte chemotaxis in vitro studies.

METHODS

Study cohort and data acquisition

Between January 1999 and March 2002, we screened 228 IgA nephropathy patients from 25 German Nephrology centers. All patients gave informed consent for CCR5 genotype analysis. Approval for the study was given by the local ethics committee on October 19, 1998 and has been conducted according to the Declaration of Helsinki principles.

Only Caucasian patients with a histologically proven primary IgA nephropathy who had been diagnosed by light microscopy and immunohistology were included in the study. Patients with secondary causes of IgA nephropathy (Henoch-Schönlein purpura, liver cirrhosis, lupus nephritis, and diabetic nephropathy) were not included. Thirty-eight patients were excluded after primary recruitment because of lack of clinical follow-up data.

The following demographic and clinical data were retrospectively assessed from the clinical records at the timepoint of recruitment: Age, gender, proteinuria (>1 g/24 hours), serum creatinine (mg/dL), and blood pressure [presence or absence of hypertension (systolic blood pressure greater than 140 mmHg) or prescription of antihypertensive treatment] all at the time point of renal biopsy. The study end point was ESRD defined through start of renal replacement therapy. Dialysis was initiated in accordance to the European Renal Association-European Dialysis and Transplantation Association guidelines [20]. Renal survival was defined as absence of renal replacement therapy during the follow-up period. Time of follow-up was the time between renal biopsy (establishing the diagnosis of IgA nephropathy) and either the last clinical follow-up of the patient with renal survival during the study period (March 2002), or the start of renal replacement therapy in those patients who reached the end point of the study. No death was reported in the follow-up period.

Detection of CCR5 Δ32 allele frequency

Genomic DNA was isolated from peripheral blood leukocytes using a commercial kit (Qiagen, Hilden, Germany). Purified genomic DNA (50 ng) was amplified by polymerase chain reaction (PCR) using the following primers: forward primer CAA AAA GAA GGT CTT CAT TAC ACC, reverse primer CCT GTG CCT TCT CTC ATG TCG. To 50 ng of genomic DNA, 29.5 µL H2O, 4 µL 10 x PCR buffer, 3 µL MgCl2 (25 mmol/L), 4 µL deoxynucleoside triphosphate (dNTP) (2.5 mmol/L), 1.5 µL forward primer (50 ng/mL), 1.5 µL reverse primer (50 ng/mL), and 0.5 µL Taq DNA Polymerase (5 U/µL) (Promega, Madison, WI, USA) were added. PCR was run for 32 cycles using the following temperature profile: denaturation at 95°C for 10 seconds, annealing for 20 seconds at 60°C, and extension for 30 seconds at 72°C. The length of the amplified fragment was 215 bp for the wild-type and 183 bp for the CCR5 Δ32 allele. The PCR fragments were separated on a 1.5% agarose gel and patients classified as weight (+/+) weight/Δ32 (+/−), and Δ32/Δ32 (−/−). Genotypes of random samples were determined by automatic DNA sequencing and showed results identical to the gel electrophoresis analysis (data not shown).

Chemotactic assays

To evaluate the effects of the CCR5 Δ32 mutation on chemotaxis we analyzed the chemotactic potency of the CCR5 chemokine ligands regulated upon activation, normal T cell expressed and secreted (RANTES), macrophage inflammatory protein (MIP)-1α, MIP-1β, and monocyte chemoattractant protein (MCP)-1 (R&D Systems, Minneapolis, MN, USA) on freshly isolated human monocytes from CCR5 wild-type, heterozygous, and homozygous Δ32 carriers (3 × 10⁶ cells/mL) in Boyden microchambers [21]. The number of migrating monocytes was used as a parameter to estimate the chemotactic response.

Statistical methods

Results are expressed as mean ± SD unless stated otherwise. Statistical significance was defined as P < 0.05 or in case of k comparisons P < 0.05/k (Bonferroni adjustment).
To compare continuous variables of two distinct groups, we applied the Mann-Whitney test. For comparison of nominal variables, the \( \chi^2 \) test was used.

To test whether the CCR5 \( \Delta 32 \) allele frequency in the IgA collective was lower than in the healthy control group, we compared the two groups using the two-sided Fisher's exact test.

The influence of the CCR5 \( \Delta 32 \) genotype on renal survival of patients with IgA nephropathy was analyzed by Kaplan-Meier plot and Log-rank test.

The effects of the CCR5 \( \Delta 32 \) genotype, serum creatinine, proteinuria, hypertension, gender, and age at the time of biopsy on renal survival were evaluated by univariate Cox proportional hazard analysis. Covariates with \( P < 0.05 \) in univariate analysis were entered into multivariate Cox proportional hazard analysis. For multivariate analysis forward conditional entry was used. \( P \) values were derived by the Wald \( \chi^2 \) test.

Differences in chemotaxis between the three groups (CCR5\( ++/\), \( +/\Delta 32 \), \( \Delta 32/\Delta 32 \)) were compared by Kruskal-Wallis test with post hoc analysis by Mann-Whitney test.

For all statistical survival analyses, the genotype was defined as presence or absence of the CCR5 mutated \( \Delta 32 \) allele. Statistical analyses were performed using the statistic program SPSS 10.1.3.

RESULTS

**CCR5 genotyping**

Presence of the CCR5 \( \Delta 32 \) genotype was determined by PCR (Fig. 1). For the 190 IgA nephropathy patients included in the study the genotypes were as follows: 158 (83.2%) patients had a CCR5 wild-type, 29 (15.3%) were heterozygous carriers of the mutation, and three patients had a homozygous CCR5 \( \Delta 32 \) genotype (1.6%). The allelic frequency of the \( \Delta 32 \) deletion was therefore 0.092.

Thirty-eight patients were excluded from the study for lack of clinical follow-up data. The CCR5 \( \Delta 32 \) genotype distribution was as follows: 32 (84.2%) patients had a CCR5 wild-type genotype, six (15.8%) were heterozygous carriers, and no patient (0%) had a homozygous CCR5 \( \Delta 32 \) genotype. The \( \Delta 32 \) allelic frequency in this group was 0.079.

To analyze the distribution of the CCR5 \( \Delta 32 \) allele in a control collective, a random selection of 651 German healthy blood donors was screened after confirmed consent to analyze their CCR5 genotype. Of those, 498 (76.5%) were CCR5 wild-type carriers, 144 (22.1%) were heterozygous for the \( \Delta 32 \) mutation, and nine (1.4%) individuals showed homozygosity. Genotype distribution in both collectives was according to the Hardy-Weinberg equilibrium. There was a nonsignificant tendency for an increased allele frequency in the control group (0.124) in comparison to the IgA collective (\( P = 0.08 \) by two-sided Fisher's exact test).

**Demographic and clinical data of the study cohort**

Table 1 shows an overview of the demographic and clinical data assessed [age in years, gender, hypertension, serum creatinine level, and proteinuria (>1 g/24 hours)] at the time point of renal biopsy, as well as the follow-up in months, and the percentage of ESRD development. At the time point of renal biopsy, the characteristics of the patients in the two groups were similar, with the exception of a significantly higher serum creatinine level in the CCR5 wild-type group. The percentage of ESRD development was also higher in the CCR5 wild-type group. The follow-up time in months was significantly longer in the CCR5 \( \Delta 32 \) patient group.

**Effects of the CCR5 genotype on the clinical course in IgA nephropathy**

Analysis of cumulative renal survival by Kaplan-Meier plot with subsequent Log-rank test showed a significantly longer survival in the group of IgA nephropathy patients carrying the CCR5 \( \Delta 32 \) genotype (\( N = 32 \)) in comparison to the wild-type group (\( N = 158 \)) (\( P < 0.001 \)) (Fig. 2).

The prognostic value of serum creatinine level, 24-hour proteinuria, hypertension, age, gender, and CCR5 genotype on renal survival in patients with IgA nephropathy was evaluated by univariate Cox proportional hazard analysis. Our results confirm earlier studies showing that the serum creatinine value at the time of biopsy is the major prognostic factor for clinical outcome. Interestingly, in our collective of IgA nephropathy patients the CCR5 \( \Delta 32 \) genotype is the second most important prognostic factor for renal survival. Age, hypertension, gender, and proteinuria (>1 g/24 hours at the time of renal biopsy) were not prognostic factors in univariate analysis.

Serum creatinine and CCR5 \( \Delta 32 \) genotype were entered into multivariate Cox proportional hazard analysis to test if both covariates are prognostic factors for renal survival independent of each other. Multivariate analysis by three different models (enter, forward conditional entry, and backward conditional exclusion) supported the
Table 1. Demographic and clinical data of the study cohort

|                          | CCR5 wild-type (+/+) (N = 158) | CCR5 Δ32 genotype (+/Δ32 and Δ32/Δ32) (N = 32) | Total (100%) (N = 190) | P value
|-------------------------|---------------------------------|-----------------------------------------------|------------------------|------------------------
| Age at biopsy years     | Mean 39.1 ± 14.2 (N = 146)     | Mean 40.8 ± 14 (N = 28)                       | Mean 39.4 ± 14 (N = 174) | 0.35 (NS)               |
| Gender% male            | 74.7% (N = 158)                | 78.1% (N = 32)                                | 75.3% (N = 190)         | 0.82 (NS)               |
| Hypertension at biopsy  | 74.1% (N = 137)                | 64.2% (N = 28)                                | 72.1% (N = 165)         | 0.36 (NS)               |
| Proteinuria at biopsy   | 67% (N = 133)                  | 63% (N = 27)                                  | 67.5% (N = 160)         | 0.65 (NS)               |
| Creatinine at biopsy mg/dL | Mean 2.6 ± 1.7 (N = 32)   | Mean 1.7 ± 1.5 (N = 29)                       | Mean 2.4 ± 1.9 (N = 165) | 0.001                  |
| Follow-up months        | Mean 48.1 ± 48.3 (N = 158)     | Mean 65.9 ± 54.0 (N = 32)                     | Mean 51 ± 49.6 (N = 190) | 0.042                  |
| Development of end-stage renal disease | 57.6% (N = 158) | Mean 50.5 (1–190) | Mean 51.6% (N = 190) | <0.001 |

Continuous variables are expressed as mean ± SD and median with range. Nominal variables are expressed as percentage. Numbers in parenthesis indicate the number of patients for whom the corresponding clinical data could be obtained.

![Cumulative renal survival plots](image)

**Fig. 2. Influence of the CCR5 Δ32 genotype on renal survival in patients with IgA nephropathy.** Kaplan-Meier plots of renal survival are shown in months from the date of biopsy until either end stage renal disease or the last follow up (CCR5 Δ32 genotype vs. wild-type, P < 0.001, Log-rank test).

The Δ32 genotype increased renal survival in patients with IgA nephropathy. Kaplan-Meier plots of renal survival are shown in months from the date of biopsy until either end stage renal disease or the last follow up (CCR5 Δ32 genotype vs. wild-type, P < 0.001, Log-rank test).

**Table 1. Demographic and clinical data of the study cohort**

- **Age at biopsy years**: Mean 39.1 ± 14.2 (N = 146) versus Mean 40.8 ± 14 (N = 28) vs. Mean 39.4 ± 14 (N = 174); P = 0.35 (NS).
- **Gender% male**: 74.7% (N = 158) versus 78.1% (N = 32) vs. 75.3% (N = 190); P = 0.82 (NS).
- **Hypertension at biopsy** (systolic blood pressure > 140 mm Hg): 74.1% (N = 137) versus 64.2% (N = 28) vs. 72.1% (N = 165); P = 0.36 (NS).
- **Proteinuria at biopsy**: 67% (N = 133) versus 63% (N = 27) vs. 67.5% (N = 160); P = 0.65 (NS).
- **Creatinine at biopsy mg/dL**: Mean 2.6 ± 1.7 (N = 32) versus Mean 1.7 ± 1.5 (N = 29) vs. Mean 2.4 ± 1.9 (N = 165); P = 0.001.
- **Follow-up months**: Mean 48.1 ± 48.3 (N = 158) versus Mean 65.9 ± 54.0 (N = 32) vs. Mean 51 ± 49.6 (N = 190); P = 0.042.
- **Development of end-stage renal disease**: 57.6% (N = 158) versus 21.9% (N = 32) vs. 51.6% (N = 190); P < 0.001.

**Discussion**

IgA nephropathy is the most frequent form of glomerulonephritis in the world. Initially believed to have a benign course, it is now clear that about one third of the patients with IgA nephropathy develop a progressive loss of renal function eventually leading to ESRD [3–5]. Some
conditions was standardized as 100%. Chemotaxis of monocytes toward all tested chemokines was significantly increased compared to basal (100 ng/mL) induced chemotaxis on freshly isolated monocytes was assessed in a chemotactic assay. The number of migrated M/M under basal condition 100%.

The different incidence in various ethnic populations and the heterogeneous pattern of disease progression suggest that genetic factors could also determine the natural course of IgA nephropathy [22].

Pathophysiologically the progression to ESRD in patients with IgA nephropathy is largely correlated with the infiltration of monocytes and lymphocytes into the renal tissue [6–8]. Chemokines, a family of chemotactic cytokines, are considered to be the main regulators of leukocyte trafficking under homeostatic and inflammatory conditions [9, 10]. In experimental models of glomerulonephritis, blockade of the chemokine receptor CCR5 with neutralizing antibodies or a peptide antagonist leads to a reduction in leukocyte recruitment and improvement of renal pathology [11, 24]. Studies of tissue samples from patients with IgA nephropathy revealed an increased expression of the CCR5 ligands MIP-1α and RANTES [25, 26] and demonstrated a correlation

### Table 2. Cox proportional-hazard analysis to assess the effect of the evaluated factors (serum creatinine, CCR5 Δ32 genotype, proteinuria, hypertension, gender, and age at the time of biopsy) on renal survival

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Univariate analysis Hazard ratio (95% CI); P value</th>
<th>Multivariate analysis Hazard ratio (95% CI); P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum creatinine</td>
<td>1.52 (1.38–1.67); &lt; 0.001</td>
<td>1.52 (1.38–1.68); &lt; 0.001</td>
</tr>
<tr>
<td>CCR5 Δ32 genotype</td>
<td>0.29 (0.13–0.62); 0.001</td>
<td>0.23 (0.09–0.57); 0.002</td>
</tr>
<tr>
<td>Proteinuria &gt;1 g/24 hours</td>
<td>1.06 (0.66–1.7); 0.83</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.84 (0.5–1.42); 0.51</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>1.13 (0.72–1.77); 0.61</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.01 (0.99–1.02); 0.48</td>
<td></td>
</tr>
</tbody>
</table>

P values were derived by the Wald χ² test. The univariate Cox proportional-hazard analysis of the variables serum creatinine, CCR5 genotype, proteinuria, hypertension, gender, and age revealed significant influence on renal survival for serum creatinine and CCR5 Δ32 genotype. Multivariate analysis revealed a high independent prognostic value for creatinine at the time of biopsy as well as the CCR5 Δ32 genotype.
between the intensity of renal infiltration of CCR5-positive leukocytes and disease progression [12]. These data suggest a possible role of CCR5-bearing mononuclear cells attracted by locally produced MIP-1α and RANTES in the pathogenesis of IgA nephropathy.

A recently identified common 32 bp deletion mutation in the CCR5 gene (CCR5 Δ32) was shown to lead to a non-functional receptor. Heterozygous CCR5 Δ32 carriers have a reduced CCR5 expression on mononuclear cells [27] and when infected with HIV might have a slower progression rate to acquired immunodeficiency syndrome (AIDS) [28]. Since the high allele frequency in the European population cannot be explained through random genetic drift, this finding suggests an unknown selective advantage of allele carriers [13]. Previous reports demonstrated a reduced CCR5 Δ32 allele frequency in patients with asthma [17]. A significantly lower prevalence of the CCR5 Δ32 genotype was detected in patients with severe compared to nonsevere forms of rheumatoid arthritis [18]. Our findings of a relatively reduced CCR5 Δ32 allele frequency in patients with IgA nephropathy in comparison to the healthy control group may underscore the importance of these findings. One might speculate that CCR5 Δ32 carriers have a decreased risk to develop IgA nephropathy or that the Δ32 allele favors less severe forms of IgA nephropathy, which in many cases might not be diagnosed (by renal biopsy) due to lack of clinical risk factors.

In this study, we investigated the effect of the CCR5 Δ32 genotype on the clinical outcome in IgA nephropathy. Comparison of cumulative renal survival with the Log-rank test demonstrated that renal survival of IgA nephropathy patients with the CCR5 Δ32 genotype was significantly longer compared to patients with the CCR5 wild-type genotype ($P < 0.001$).

We used the multivariate Cox proportional hazard model to adjust for comorbid risk factors. The results demonstrated that the CCR5 Δ32 mutation is an independent factor associated with a significantly reduced risk of ESRD among patients with IgA nephropathy. Indeed, the CCR5 Δ32 genotype was the second most important independent prognostic factor for renal survival (after the serum creatinine level). Unexpectedly, the presence of hypertension at the time of biopsy, proteinuria (>1 g/24 hours), and gender had no significant influence on renal survival in our study. This is likely to be due to the high incidence of these risk factors in our population (about 72% of the patients had hypertension, at least 67% had proteinuria, and more than 75% were male), as well as to the use of nominal values for hypertension and proteinuria, which might not allow a statistical discrimination.

Due to the small number of homozygous CCR5 Δ32 IgA nephropathy patients in our study (three of 190 patients), a statistical analysis of this subgroup cannot be performed. However, the fact that two of the three patients in this group developed ESRD indicates that the absence of a functional CCR5 does not completely protect against disease progression in IgA nephropathy.

Interestingly, the protective effect of the CCR5 Δ32 genotype was most prominent in the first years following renal biopsy. There might be several reasons for this phenomenon. In IgA nephropathy and other forms of glomerulonephritis, the inflammatory cell-mediated phenotype is probably more important in early disease stages. Since our in vitro studies revealed a significantly reduced chemotaxis of monocytes from CCR5 Δ32 carriers, it is tempting to assume that the protective effect is mediated by a reduced recruitment of CCR5-bearing mononuclear cells into the renal tissue, leading to an attenuated inflammatory response in the kidney. When time progresses, the inflammatory phenotype changes to a more fibrotic picture, with renal nephrosclerosis. At this stage, which occurs 5 to 10 years after onset of the disease, inflammatory mediators may no longer affect the progressive nature of IgA nephropathy.

This is the first study to demonstrate an independent protective role of the CCR5 Δ32 genotype for the clinical outcome in a cohort of patients with IgA nephropathy who showed a high progression rate to ESRD. Because of the specific characteristics of our study group and the retrospective nature of our study, the results should be considered exploratory. Future studies will be necessary to confirm the protective effect of the CCR5 Δ32 genotype in IgA nephropathy in other populations.

APPENDIX

The following German Nephrology center participated in this study. The number of provided patients by each center is enclosed in parentheses.

Universitätsklinikum Hamburg Eppendorf: U. Panzer, A. Schnei-de, O.M. Steinnmetz, U. Wenzel, P. Barth, R. Reinking, J.U. Becker, S. Harendza, G. Zahnher, U. Melchern, R.A.K. Stahl, U. Tenschert, (N = 48); Klinikum der Universität Regensburg and Klinikum der Universität München: M. Fischeder, B.K. Krämer, D. Schöndörff (N = 17); Universitätssklinikum Aachen: T. Ostendorf, J. Floege (N = 32); Nephrology Center Schlankerey Hamburg: D. Amir, I. Krenz, J. Remmcke (N = 11); Krankenhaus der Barmherzigen Brüder Trier: W.H. Boesken (N = 4); Nephrology Center Harburg: F. Bode, Arndt (N = 9); Nephrology Praxis Parchim: C. Brenning (N = 6); Nephrology Praxis Schultwiete Hamburg: T. Döl, K.O. Stenger (N = 6); Nephrology Praxis Reinebeck: P. Färber, D. Göuer (N = 5); Universitätssklinikum Dresden: T. Franz (N = 7); Nephrology Center Alter Teichweg Hamburg: S. Grosser, A. Kühns (N = 10); Klinikum Nord Hamburg: Henrici, H. Liebau (N = 2); Nephrology Praxis Boffzen: S. Kien-Schneider (N = 1); Nephrology Center Langenhorn Hamburg: P. Kusche (N = 4); Zentralkrankenhaus St. Jürgen Straße Bremen: A.E. Lison (N = 1); Franziskus Hospital Bielefeld: V. Luft (N = 2); Nephrology Praxis Langenhagen Hannover: N. Lustenberger, H. Schmidt-Günter, G. Obere (N = 10); Dialysis Praxis Waldweg Hamburg: S. Mees, H. Wüns (N = 5); Nephrology Praxis Hameln: D. Schumann (N = 1); Nephrology Praxis Welsensee Berlin: G. Schwietzer (N = 1); Nephrology Praxis Fürstenzett: A. Stark (N = 1); Nephrology Praxis Uelzen: S Wedel, R. Weitzell (N = 5); St. Barbara Hamm Klinik: H. Pfeiferer (N = 1); and Stadtkrankenhaus Cuxhaven: Insellmann (N = 1).
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REFERENCES