Inflammation and malnutrition in patients with chronic kidney disease: Focus on quality of life
Associations and possible roles of red blood cell distribution width in kidney transplant recipients

Thesis

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Budapest
2015

INTRODUCTION

Chronic Kidney Disease (CKD) and End Stage Renal Disease (ESRD) prevalence is increasing and occurrence of it has reached up to 10-13% in the western countries. According to morbidity and mortality data it can be considered as epidemic disease. Approximately 30% of people suffering with CKD are unaware of their condition. This lack of unawareness can lead to under treatment and hence it is not a surprise that thousands of people affected with CKD die every year, mostly due to CKD related cardiovascular disease. In recent years, life expectancy of patients undergoing renal replacement therapy has increased, making CKD a life-long chronic disease with new challenges to the attending physicians. Moreover, because of the diversity of patients developing CKD, individual risk prediction is important and necessary and can assist in developing individualized treatment strategies to improve each patient's survival and well-being while living with the disease. CKD symptoms, comorbid conditions and maintenance renal replacement therapy have limited the CKD patient's quality of life, but the traditional medical evaluations have not measured them correctly and do not accurately reflect the patient's condition. In modern medicine, obtaining the patients' opinion on their health status and treatment, including the measurement of their health-related quality of life (HR-QoL), is important when designing appropriate life extending treatment strategies.

CKD has an impaired effect on the patients' and their relatives' physical, mental and social quality of life. Patients on maintenance dialysis are more likely depressed, stressed, and worried about their livelihood difficulties, family conflicts and losing their independence. Conversely, kidney transplant recipients have reported to have a higher quality of life compared to maintenance dialysis counterparts when using most domain of HR-QoL evaluation. In studies where the Short-Form-36 (SF-36) questionnaire was used instead, kidney transplant recipients reported quality of life was not significantly better than dialyzed patients.

Nutritional and body composition related adverse changes are closely associated with quality of life and also highly prevalent in patients with chronic disease. According to our current knowledge, renal transplantation is the optimal treatment for patients with late stage CKD, however even kidney transplant recipients are not protected from the appearance of nutritional alterations. Protein-energy wasting (PEW) has been used since 2008 as a measure for the malnutrition and inflammation syndrome. PEW is associated with frequent hospitalization, higher morbidity, mortality, and worse quality of life. Therapy of PEW does not have any gold standard guidelines. In clinical practice it is typically hard to evaluate the degree of PEW. The presence of PEW is unequivocal only in critically ill, cachexic patients, when usually it is too late for the effective therapy. Kalantar-Zadeh and et al. have developed

an easy to use, semi-quantitative tool for the estimation of the degree of PEW in dialyzed patients called the malnutrition-inflammation scale (MIS). According to study results evaluating the effectiveness of MIS, it has a comprehensive ability for measuring clinical status, and its score is associated with patient hospitalization, mortality, nutrition and inflammatory and anemia status, as well. Association between MIS and quality of life has not yet been evaluated in kidney transplant recipients. Our study was designed to accurately evaluate the relationship of MIS and QoL in kidney transplant recipients.

Knowledge regarding the evaluation and treatment of CKD is not only important for assigning renal replacement therapy, but also due to the fact that the kidney disease is an independent predictor of morbidity and mortality. In kidney transplant recipients the highest cause of death is cardiovascular mortality (40-55%). The adverse effects of CKD on cardiovascular status appear early on in CKD and the rate of progression of cardiovascular disease in CKD patients is also higher compared to general population.

Overall, short-term mortality of renal transplant patients is well predictable given already existing tools. However, for individual risk prediction there is a need for development of more accurate instruments. Red blood cell distribution width (RDW) has recently been identified as a new, strong and independent predictor of mortality and morbidity in patients with chronic heart failure. RDW seems to be a prognostic factor for all-cause mortality in patients with heart failure regardless of hemoglobin levels and anemia status. Given its success as a predictive marker in heart failure patients, we decided to evaluate RDW as a risk factor and prognostic marker for outcomes in kidney transplant patients.

Despite the fact that many previous publications have corroborated the reported association of RDW and mortality, the reasons underlying this association is still unknown. A large epidemiological study has found an association between RDW and estimated glomerular filtration (eGFR) and in a group of outpatients. We hypothesize that the relationship of RDW and eGFR may explain RDW’s association with higher mortality, and seek to examine these associations in a cohort of kidney transplant recipients.

AIMS AND HYPOTHESES

Association of Health-related Quality of Life with Malnutrition and Inflammation score in kidney transplant recipients.

In ESRD patients PEW is associated with decreased QoL. According to results from our previous studies, MIS scale is an appropriate tool to measure PEW, but the relationship between MIS with QoL is not known. The aim of our study was to evaluate the association of nutritional and inflammation status and HR-QoL in kidney transplant recipients.

Our hypotheses:

- MIS, as an indicator of nutritional and inflammation status, is independently associated with HR-QoL in renal transplant patients after adjustment for relevant confounding factors.

Association of red blood cell distribution width with graft function in kidney transplant patients.

Previous studies have shown that elevation of RDW is affected by many factors, but the exact mechanism responsible for increasing RDW is still unknown. Prior analysis in outpatient registry data showed that impaired kidney function is related to elevated RDW levels. However, there have been no subsequent follow-up studies in renal transplant patients, which can corroborate this association.

Our hypotheses:

- RDW is related to comorbidities, nutritional and inflammatory status, and it is independently associated with estimated graft function in renal transplant patients.

Prospective analysis of the association of RDW and mortality in renal transplant patients

In kidney transplant recipients the most common cause of death is cardiovascular mortality, and in various groups of cardiovascular disease patients, higher RDW values have been associated with an increased mortality risk. We have analyzed the association of RDW and mortality in our prospectively followed renal transplant patients, and evaluated the possible use of RDW in risk prediction.

Our hypotheses:

- In a prospective cohort of renal transplant patients, RDW is independently associated with mortality;
- In a prospective cohort of renal transplant patients, RDW has its own and additional prognostic value to mortality risk prediction.

METHODS

Study population and collection of data

We invited all prevalent kidney transplant recipients, 18 years of age or older (n=1214), who were followed at a single transplant outpatient clinic at the Department of Transplantation and Surgery at the Semmelweis University Faculty of Medicine, Budapest, Hungary on December 31, 2006 to participate in a prospective, observational study (Malnutrition-Inflammation in Transplant - Hungary Study [MINIT-HU Study]). We began following the study cohort in February of 2007 with annual data collection until November of 2009. We subsequently followed patients only for mortality outcomes after until December 2011.

The total population of 1214 patients was invited to participate in our study. Inclusion criteria were: age of at least 18 years and and written consent of participation after providing detailed written and verbal information regarding the study. The exclusion criteria were: acute rejection within the last 4 weeks, current hospitalization, transplantation in the previous 3 months, or acute infection or bleeding. After the application of these criteria, there were 1198 patients who were eligible to participate in our study.

We randomly selected 150 patients (‘‘study sample’’) and invited them to participate in our sub-study on QoL and MIS. We used the simple random sampling strategy previously described in the SLeep disorders Evaluation in Patients after kidney Transplantation (SLEPT) Study to identify these patients (SPSS 15.0 was used for random patient selection). Of the 150 randomly selected patients; 50 (33%) refused to participate and 100 transplant patients (participants) were included in the final study population for this analysis.

The study group used to evaluate RDW was selected from patients present during the second year (2008) of follow-up. Of the potential 1214 patients in our cohort, 205 (17%) refused to participate in this sub-study at the baseline data collection, 71(7%) patients refused to continue participation or were lost to follow-up after the first year, 115(10%) patients had an outcome event during the first year and were therefore excluded, 2(<1%) were missing data on RDW, and 82(10%) were not included in the primary analyses because they were receiving ESA therapy. There were therefore 723 ESA untreated patients for our primary analyses. The 82 excluded patients receiving ESA therapy were then added back for a secondary analysis.

Socio-demographic data and details of medical history were collected at enrollment when information about age, gender and menopause status, highest education level achieved, marital status (‘‘married’’ or ‘‘other’’ including single, widow and divorced), occupational

status (‘‘part or full time job’’ or ‘‘other’’ including retired, unemployed or else),etiology of CKD, and transplantation-related data, including immune suppressant medication use and comorbidities measured with the modified Charlson Comorbidity Index (CCI), were obtained. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. All participants were asked to fill out the depression questionnaire, Center for Epidemiologic Studies – Depression (CES-D). Assessment of MIS was carried out by our workgroup during a short interview. Standard blood pressure measurements were collected three times and mean values were calculated. Laboratory, anthropometrical (body weight, height, and abdominal circumference), MIS and CES-D scale data were collected all on the same day of patient visit. The QoL questionnaire was assessed the in the following weeks during a sleep examination. Moreover, in the total follow-up time of 5 years, outcome data were collected, regarding to the time of death or loss of graft function. Information about cause and consequences of graft failure and cause of death were not obtained.

Ethics approval of research

The study was approved by the Ethics Committee of the Semmelweis University. MINIT-HU (49/2006) and SLEPT (4/2007) study. The study was carried out according to ICP Good Clinical Practices Guidelines and Helsinki-declaration. Before enrollment, patients received detailed written and verbal information regarding the aims and protocol of the study and gave written consent to participate.

Malnutrition-inflammation score

We used the malnutrition-inflammation score (MIS), recently called Kalantar Score, developed and published by Kalantar-Zadeh et al., to assess protein-energy wasting. The MIS has 10 components, each with four levels of severity, from 0 (normal) to 3 (severely abnormal). The sum of all 10 MIS components ranges from 0 (normal) to 30 (severely malnourished); a higher score reflects a more severe degree of malnutrition and inflammation status. First questions regarding to patient anamnesis: changes in weight in the last 3-6 months, appetite, gastrointestinal problems, physical strength and comorbidities additionally to kidney disease was assessed. Than the subjective global assessment (SGA) score related muscle and body fat alterations were measured. Patients received higher points on the MIS when their BMI was below normal range. The last part of the questionnaire is to determine the scale points with regards to serum albumin and transferrin. All SGA was performed by an

experienced physician (ME Czira) according to conventional SGA guidelines specified elsewhere.

Assessment of quality of life

Health-related quality of life (HR-QoL) was assessed with the commonly used KDQOL-SF™ questionnaire. We have recently provided evidence that most of the subscales of the Hungarian KDQOL-SF™ are psychometrically sound and reliable both in dialyzed and transplanted populations. The KDQOL-SF™ includes the Medical Outcomes Study Short Form- 36 generic core (SF-36) and several multi-item scales targeting quality of life concerns with special relevance for patients with CKD. The generic dimensions consist of 8 multi-item measures of physical and mental health status: ‘‘Physical functioning’’; ‘‘Limitation in role function for physical reasons’’; ‘‘Bodily pain’’; ‘‘General health perceptions’’; ‘‘Energy/fatigue’’; ‘‘Social function’’; ‘‘Limitation in role function for emotional reasons’’ and ‘‘Emotional well-being’’.

The disease-specific domains, which are used in this analysis, focus on particular health-related concerns of individuals with kidney disease: ‘‘Symptoms/problems’’; ‘‘Effects of kidney disease on daily life’’; ‘‘Burden of kidney disease’’ and ‘‘Sleep’’. Scores for each item are computed in order to gain a potential range from 0 to 100 within each dimension/domain, with higher scores indicating better HR-QoL. The physical and mental composite scores of the KDQOL-SF™ questionnaire were estimated from the general US population. Composite scores around 50 mean an equal quality of life with the US general population. Below 50 is worse, above 50 means better quality of life than the US general population.

In the present analysis, scores obtained with the Center for Epidemiologic Studies-Depression (CES-D) questionnaire were used to describe the severity of depressive symptoms. The Hungarian version of the CES-D scale contains 20 questions about depressive symptoms, focusing on the last 2 weeks. The score range is between 0-60. Higher CESD score is associated with higher prevalence of depressive symptoms. In patients with kidney disease, the threshold for clinical significant depression is 18.

Laboratory data

All laboratory data was measured during the study visits in a fasting state and included (among others): RDW, blood hemoglobin concentration (Hb), serum C-reactive protein (CRP) and creatinine, blood urea nitrogen (BUN) and serum albumin levels. Ferritin level was determined as a marker of total iron stores. To determine functional iron availability transferrin levels, soluble transferrin receptor, serum iron and total iron binding capacity were

also measured. RDW was measured as part of a standard complete blood count measurement. Serum samples were also collected at the time of the baseline assessment and stored at -70°C for future use.

Comorbid conditions

We used the modified Charlson Comorbidity Index, which is a weighted scoring system based on the presence or absence of each of 17 variables. Earlier, it has been reported that the Charlson Comorbidity Index was a predictor of survival in kidney transplant patients. Because one of the variables is the presence of moderate to severe kidney disease, the minimum score for all patients with end-stage renal disease is 2. Thus, in patients with end-stage renal disease, scores range starting from 2. Additionally we collected the data of hypertension, cardiovascular disease, sever neurology disease, moderate or severe nonspecific lung disease and about chemotherapy.

Transplantation-related data and donor characteristics

Transplant-related data was extracted from the medical records and included current medications (including current immunosuppressive treatment), transplant history, i.e. time elapsed since the date of transplantation, length of time on dialysis, type of allograft, history of treated acute rejection (s) after transplantation, human leucocyte antigen (HLA) mismatch, panel reactive antibodies titer (PRA), cold ischemia time, donor age and gender, and history of delayed graft function. Delayed graft function was defined as, at least one dialysis treatment on the first week of transplantation

Immunosuppressive therapy

Standard maintenance immunosuppressive therapy in stable patients generally consisted of prednisolone, either cyclosporine A micro emulsion formulation (CsA) or tacrolimus, combined with mycophenolate-mofetil (MMF) or azathioprine or sirolimus.

Statistical analysis

Descriptive statistics

Descriptive data were summarized using proportions, means ± standard deviation (SD) or medians (interquartile range) as appropriate.

Association of MIS and QoL

In our analysis we divided the 100 randomly selected patients based on MIS median (3). Continuous variables were compared using Student's t-test or the Mann–Whitney U–test, and categorical variables were analyzed with chi-square test. Correlation analyses were performed using Pearson's or Spearman's correlation. Since the distributions of QoL domains

were not normal, we transformed these variables (transformed QoL domain = ln (original QoL domain scores +10)). For multivariate analyses, multiple linear regressions were applied. In these models, we used the transformed QoL variables as dependent variables. Variables were included in the multivariate models based on theoretical considerations and also on the results of the bivariate analyses. Regarding to the role of depression in QoL, we made a separate multivariate model including the results of CESD score.

Association of RDW and kidney function

In our analyses, patients (n=805) were allocated to one of three groups according to their tertile RDW levels [1 tertile: <13.3% (n = 215); 2 tertile: 13.3–14.0% (n = 258); 3 tertile ≥14.1% (n = 252)]. Across RDW tertiles, variables were compared using analysis of variance (ANOVA) test for homogeneity to evaluate differences across groups, and using test for trend to evaluate if the linear trend or ordering increased or decreased covariate levels across groups. For continuous variables, Pearson's or Spearman correlations were used based on the distribution of the data to report correlation of with RDW. From our primary analyses, patients treated with erythropoietin agent (ESA) were excluded (n=82) resulting a final group of 723 patients. We performed sensitivity analysis, where the ESA treated patients were also included. We further examined associations in subgroup analyses according to apriori selected relevant factors.

Logistic regression models were used to identify markers associated with elevated RDW (>14.0%). Linear regression and multivariate-adjusted regression models were used to analyze the association between RDW and eGFR. The variables entered in the multivariate-adjusted models were selected based on theoretical considerations; we included predictors in the models that were known to be associated with RDW levels based on external evidence and clinical experience, and were available in our database.

Association of RDW and mortality

In the first 3 years of follow-up, data collection of medical data was repeated annually. After the 3rd year of follow-up, there was no further medical data collection only information about survival and dialysis treatment. After the measurement of RDW in the second year (2008), the mean follow-uptime was 32 months (median (IQR): 35.2 (3.3)). Primary outcomes evaluated were all-cause mortality and loss of graft function (start of dialysis therapy). Date of death or start of maintenance dialysis was collected from medical records and was verified with the data from National Health Insurance Fund of Hungary (OEP) and Central Statistical Office (KSH).

After crude mortality analysis, the association between baseline RDW level and all-cause mortality was assessed using Cox proportional regression analysis and Kaplan–Meier plots with log rank test. Proportional hazards assumptions were tested using scaled Schoenfeld residuals. The variables entered in the multivariate-adjusted models were selected based on the theoretical considerations; namely, we included predictors in the models which were known to be associated both with RDW levels and with mortality based on external evidence and clinical experience, and which were available in our database.

As sensitivity analysis, we also examined associations using the outcome of death with functioning graft (no prior graft failure). Because death with functioning graft and transplant loss are competing events, we used competing risk models to better analyze the risk of death with functioning graft. Our event of interest was the death with functioning graft, and the competing event was the graft failure. In our study, we used the Fine and Gray model, which extends the Cox proportional hazards model to competing-risks data by considering the sub-distribution hazard. As an additional sensitivity analysis, we also repeated our main analysis on also in a cohort including patients receiving ESA treatment.

To evaluate the predictive value of RDW, receiver operating characteristics (ROCs) analysis was performed and the area under the ROC curve (AUC) was calculated. To better interpret the potential clinical utility of RDW, we conducted reclassification analysis to examine the effect of using different RDW cut-off points (5, 15 and 30 %) to predict all-cause mortality. Due to the relatively limited number of events and the short follow-up time, the estimated probabilities of all-cause mortality by a logistic regression model was divided into 4 risk categories. (low risk: <5%, mid-low risk: 5–14%, mid-high risk: 15–29%, and high: >30%). Reclassification improvement was quantified using the net reclassification improvement (NRI) statistic. Goodness of fit was estimated using likelihood ratio, Harell's C coefficient method and the AIC (Akaike information criterion), which includes the statistical goodness of fit and the numbers of parameters required to achieve the degree of fit.

RESULTS

Association of malnutrition-inflammation scale with health-related quality of life in kidney transplant recipients

Basic characteristics of the 100 participating transplant patients in the MIS-QoL sub-study were similar to the characteristics of the total clinic population. We therefore think the randomized study sample is representative of the total kidney transplant recipient cohort.

Analyses of composite scores of KDQoL-SF^{TM} and MIS using cubic spline method were performed. These analyses revealed a linearly increasing, ‘‘dose–response’’ relationship between the composite scores of KDQoL-SF^{TM} and MIS. QoL, in almost all assessed dimensions, was worse in patients with high MIS score (above median) compared to the low MIS score group (based on MIS median). Additionally, QoL scores showed significant negative correlation with MIS score along almost all domains. However, observed differences between MIS score groups were clinically significant for both the general domains assessing physical health status (Energy/fatigue, Bodily pain) and also for the mental aspects (Mental health) of QoL as assessed by the SF-36 generic instrument. The kidney disease-targeted domains of the KDQOL-SF^{TM} questionnaire include a subscale related to sleep. Similarly to other QoL dimensions, patients with high MIS had a worse score on this subscale, than patients with low MIS, but this difference did not reach statistical significance.

As some of the kidney disease-targeted subscales of both the Hungarian and the original English KDQOL-SF^{TM} instrument had suboptimal psychometric properties, only the reliable and valid kidney disease-specific QoL domains were used in this analysis. The Associations of MIS with disease-specific Qol domains were similar to those with the generic part of the QoL questionnaire; where higher MIS score were linearly associated with lower QoL domain scores.

To analyze whether MIS was robustly and independently associated with impaired QoL, multiple linear regression models were built with selected and transformed QoL scores as the dependent variables. In addition, several clinical and socio-demographic covariates (age; gender; eGFR; dialysis vintage; Charlson Comorbidity Index score and occupational status) were used in the models in addition to the MIS. Remarkably, MIS was independently associated with almost all analyzed QoL domains even after controlling for the important covariates. After adjusting for CES-D score, the association between MIS and QoL scores still remained significant in some, but not all domains.

Associations of red blood cell distribution width with kidney function in renal transplant patients

Of the potential 1214 eligible patients in the first year of assessment, the baseline study sample included 993 patients. Patients undergoing ESA treatment were excluded from primary analyses and RDW was missing for two patients, resulting in a cohort of 723 ESA untreated patients for our primary analyses.

Patients with the highest RDW values had worst kidney function (63.9±19.3 vs. 53.6±18.9 vs. 47.1±18.6 ml/min; $p<0.001$), were older (46±14 vs. 52±12 vs. 55±11 years;

p<0.001), had more time elapsed since ESRD diagnosis (110 (73) vs. 121 (79) vs. 126 (103) months; p=0.005) and had more comorbid conditions. Patients with the highest RDW values also had lower hemoglobin levels (142±14 vs. 140±14 vs. 139±16 g/L; p=0.016) and more iron deficiency. They also seemed to have higher BMI values (26.4±4.6 vs. 27.9±5.0 vs. 27.7±4.7 kg/m2; p=0.006) and significantly higher measures of central obesity (abdominal circumference) (96±13 vs. 100±14 vs. 102±13 cm; p<0.001). Additionally, patients with highest RDW had worse nutritional and inflammatory profile, as indicated by lower serum albumin (41.5±3.6 vs. 41.2±3.6 vs. 39±3.9 g/L; p<0.001) and higher CRP levels (2.4 (2.8) vs. 2.8 (3.8) vs. 4.0 (6.2) mg/L; p<0.001) mg/L; p<0.001). Furthermore, serum phosphate levels (1.01±0.19 vs. 1.05±0.21 vs. 1.06±0.22 mmol/L; p=0.038) and intact parathyroid hormone levels (68.1 (29.4) vs. 78.6 (54.5) vs. 90.5 (82.0) pg/mL; p<0.001) were higher in the highest RDW tertile. ESA treated patients, who were excluded from our principal analyses, were older, more anemic, more likely to be female, and had higher RDW values and worse kidney function

Associations of RDW was measured in univariate correlation with other markers where we found an inverse moderate relationship with eGFR (r= -0.382; p<0.001). Moreover, markers of iron status, nutrition, inflammation and markers of bone and mineral metabolism were also correlated with RDW.

In unadjusted linear regression analyses a 10 ml/min decrease in the eGFR was significantly associated with an increase of the RDW values (B10 ml/min decrease= 0.151; 95%CI: 0.116-0.186). This association remained significant after adjusting for age, gender, cause and duration of ESRD, comorbidities and immunosuppressive medications (B10 ml/min decrease= 0.123; 95%CI: 0.088-0.157), as well as iron markers (B10 ml/min decrease= 0.087; 95%CI: 0.054-0.120). The association remained significant after additional adjustment for nutritional and inflammatory markers (B10 ml/min decrease= 0.087; 95%CI: 0.055-0.119). Moreover, additional adjustment for indicators of bone and mineral metabolism in the final model did not weaken this association (B10 ml/min decrease= 0.078; 95%CI: 0.044-0.111). The association of RDW showed and inverse, graded association with eGFR when it was modeled using linear regression in both unadjusted and fully adjusted models. In sensitivity analysis, we found qualitatively similar linear associations between eGFR and RDW when we included the 82 patients who received ESA treatment.

Unadjusted and multivariable adjusted associations of CKD stages with predicted RDW level indicated a linear and significant association of higher RDW values with lower eGFR categories. We did an additional analysis which included quadratic terms of eGFR,

which was not significant and this further confirmed that the association between RDW and eGFR was linear.

In order to further examine the association of RDW level and eGFR in consideration of potential effect modifiers, subgroup analysis was performed and significance of interaction was estimated. The association of RDW with estimated kidney function was similar in almost all examined subgroups. However, there was a significant interaction between treatment with ESA and RDW ($P_{interaction}$=0.013) for eGFR outcomes. All other tests of interactions were not statistically significant indicating no effect modification by the examined characteristics (age, gender, presence of diabetes, BMI categories, CKD stage, hemoglobin level, soluble transferrin level, endogen erythropoietin level, CRP level, albumin level, mTOR and tacrolimus use).

To identify potential risk factors of elevated RDW, we performed logistic regression analyses on relevant markers modeling risk of elevated RDW values (i.e., RDW > 14%, reference is ≤14%). In the fully adjusted models, the risk of elevated RDW was associated with each 10 ml/min decrease of eGFR ($OR_{10ml/min\ decrease}$: 1.267; 95%CI: 1.121-1.431; p<0.001). Patients with CKD stage 3 had an almost two-fold higher odds of having elevated RDW values ($OR_{eGFR\ 30\text{-}60ml/min}$: 1.995; 95%CI: 1.271-3.131; p=0.002), as compared patients who had eGFR levels higher than 60 ml/min. Moreover, patients with CKD stage 4 or 5 had even higher risk of having elevated RDW values ($OR_{eGFR\ <\ 30ml/min}$: 3.334; 95%CI: 1.557-7.138; p<0.001). In addition, older age, male gender, longer ESRD vintage, use of mTOR, lower serum albumin and higher inflammatory markers as surrogates of protein-energy wasting/inflammation were all predictors of elevated RDW levels

Association of red blood cell distribution width with mortality in kidney transplant patients

Patients used in the RDW and eGFR analyses were then examined for associations between RDW and mortality outcomes. In the median follow-up time of 35 months, 81 patients died who were involved in our primary analysis (n=723) and there were no missing data regarding to patient outcome. Crude all-cause mortality rate, including patients with or without a functioning graft was 39.3/1000 patient/year (95% Confidence interval (95% CI: 31.6-48.9). The unadjusted mortality rate was significantly higher in patients with elevated (above median) RDW. (crude mortalty rate was 67.4 (95% CI:54.1-84.1) vs. 20.5 (95% CI:13.5-31.1) /1000 patient/years in patients with lower RDW).

In unadjusted Cox proportional regression analyses, a 1 % higher RDW level significantly was significantly associated with all-cause mortality [(HR1 % increase = 1.63;

95 % CI 1.41–1.89) and (HR[median = 2.74; 95 % CI 1.68–4.48)]. This association remained significant after adjusting for age, gender, eGFR, iron markers [(HR1 % increase = 1.56; 95 % CI 1.28–1.90) and (HR[median = 1.88; 95 % CI 1.11–3.20)] and also after additional adjustment for inflammatory markers [(HR1 % increase = 1.52; 95 % CI 1.23–1.89) and (HR[median = 1.56; 95 % CI 0.90–2.67)]. Moreover, adjustment for additional relevant variables in the final model did not weaken this association [(HR1 % increase = 1.60; 95 % CI 1.27–2.02) and (HR[median = 1.33; 95 % CI 0.76–2.35)]. In sensitivity analyses, we found qualitatively similar results also when we analyzed the total population, including the 82 patients who received ESA treatment. For further confirmation, we built competing risk regression models, where death with functioning graft (n=66) was the event of interest and graft-loss (n=56) was considered as competing event. We did not find similar results to the all-cause mortality analyses.

As a verification of our previous findings we analyzed the RDW associations in various patient subgroups. The association with mortality was similar in almost all examined subgroups. In our analysis, only gender ($P_{interaction}$ = 0.031) and CRP ($P_{interaction}$ = 0.022) showed an interaction with RDW, and all other tests of interactions were not statistically significant indicating no effect modification by the examined characteristics.

ROC analysis showed that the RDW has an acceptable predictive capability with the area under the ROC curve (AUC) of 0.689 for all-cause mortality among ESA untreated kidney transplant recipients. Based on this analysis, we propose a cutoff RDW of 14 % in this population. This value had a specificity of 65 % with a sensitivity of 63 %. Positive predictive value for this variable was 18 % whereas negative predictive value was 93 %. The C-statistic, AIC and likelihood ratio improved after including RDW as an independent variable in the predictive models, although the ROC comparison showed only a slight numeric increase in the AUC (0.801 vs. 0.812; p = 0.258).

The risk prediction ability of RDW was assessed with a more clinically practical method of reclassification analysis. For all-cause mortality the net reclassification improvement (NRI) showed a significant improvement (<5 %, 5–15 %, 15–30 %, >30 %; NRI = 0.189; p<0.001) after adding RDW to the model.

CONLCUSIONS AND NEW RESULTS

In our studies, we evaluated the relationship of the malnutrition-inflammation scale with health-related quality of life and investigated the associations of red blood cell distribution width with estimated kidney function, and mortality and graft failure outcomes in kidney transplant recipients.

Below we are summarized our new findings and answered our prior hypotheses:

-MIS, as an objective measure of nutritional and inflammation status, is associated with health-related QoL in renal transplant patients;

-RDW is related with the presence of some comorbidities and measures of nutritional and inflammation status;

-RDW is closely associated with the kidney function independently from clinical and socio-demography parameters;

-In a prospective study setting RDW is, independently from many clinical and laboratory parameters, associated with mortality;

-In a prospective study setting RDW has and independent prognostic and predictive value in the mortality risk prediction in kidney transplant recipients.

In summary MIS is associated with HR-QoL not exclusively in dialysis, but in kidney transplant patients as well. QoL has an emerging role in modern health care. MIS could be a useful tool in elevating QoL with providing reliable information about PEW. We assume, that with the use of the scale, the monitoring and therapy of PEW, quality of life of renal transplant patients might elevate. The easy to use and cheap MIS and QoL questionnaires may be used in clinical practice as well.

RDW, the other focus in our studies, showed an independent association, from socio-demographical and clinical values with kidney function, which has a major role in kidney transplant outcome. Moreover, RDW has its own and additional prognostic and predictive value in mortality risk prediction. Pathophysiological reasons underlying these associations and the causes of elevated RDW are still unknown and needs further investigation. Thus we think, the proposal of using RDW in a clinical setting may be alarming. However, rates of cardiovascular mortality are still high in patients with kidney disease, and there is an urgent need to identify factors which can predict adverse outcomes such as mortality and graft failure in kidney transplant recipients. Moreover, because RDW is easily measured and already available in most lab reports, we believe that in can also be easily used in every day clinical practice to evaluate outcomes in chronic kidney disease patients.

LIST OF PUBLICATIONS

Candidate's publications Related to the Thesis

Publications accepted in international journals

Ujszaszi A, Czira ME, Fornadi K, Novak M, Mucsi I, Molnar MZ. (2012) Quality of life and protein-energy wasting in kidney transplant recipients. Int Urol Nephrol, 44(4): 1257-1268.

Ujszaszi A, Molnar MZ, Czira ME, Novak M, Mucsi I. (2013) Renal function is independently associated with red cell distribution width in kidney transplant recipients: a potential new auxiliary parameter for the clinical evaluation of patients with chronic kidney disease. Br J Haematol, 161(5): 715-725.

Mucsi I, Ujszaszi A, Czira ME, Novak M, Molnar MZ. (2014) Red cell distribution width is associated with mortality in kidney transplant recipients. Int Urol Nephrol, 46(3): 641-651.

Candidate's publications not Related to the Thesis

Publications accepted in international journals

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