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Estimation of dialysis patients' survival through combined approach of small molecule uremic markers

Jana Holmar[a*], Ivo Fridolin[a], Fredrik Uhlin[a,b], Anders Fernström[b], and Merike Luman[a,c]

[a] Department of Biomedical Engineering, Technomedicum, Tallinn University of Technology, Ehitajate tee 5, 19086 Tallinn, Estonia
[b] Department of Medical and Health Sciences, Faculty of Health Sciences, Section of Nephrology, Linköping University, Garnisonsvägen 10, 58185, Linköping, Sweden
[c] Centre of Nephrology, North Estonian Medical Centre, Sütiste tee 19, 13419 Tallinn, Estonia

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Abstract. Survival rate of dialysis patients is still alarmingly low and various factors may have in it an important role. The purpose of this study was to observe the relationship between the survival of dialysis patients and the serum level of urea, creatinine, and uric acid (UA). Serum urea and creatinine concentrations may express patient's nutritional status and muscle mass, and high UA value may refer to higher risk for cardiovascular events. The idea of combining the concentrations and removal of urea and UA into a single model for predicting the patient's outcome is introduced. The study included 33 hemodialysis patients from Linköping, Sweden and 10 from Tallinn, Estonia. Kaplan–Meier analysis was used for survival analysis. Logistic and Cox regression analysis was applied to create models for predicting patients' three-year survival. It was observed that higher serum UA is significantly related to poor survival in dialysis patients ($p = 0.026$). A reverse effect was observed in case of urea ($p = 0.095$). The level of creatinine was not related to survival ($p = 0.905$). The best logistic regression model for predicting patients' outcome included both UA and urea based parameters (Chi Square 21.0, $p = 0.0001$). Survival of dialysis patients seems to be determined by a set of causal factors and combined models may have a predictive relevance. A possibility for automatic online monitoring of small molecule uremic markers is proposed. Since the number of participating patients was small, larger studies including more patients and testing the models in independent validation cohort is the future goal.

Key words: dialysis, survival, prediction models, urea, uric acid, creatinine.

1. INTRODUCTION

It has been reported that the mean life expectancy of a hemodialysis patient is less than 3 years [1]. Therefore, markers and methods for patient outcome estimation are highly longed for. According to Clinical Practice Guidelines [2], dialysis quality is estimated via calculating urea reduction ratio (URR) or Kt/V, causing urea to be the most common marker for dialysis quality assessment. However, there is a shortage of evidence that show direct biologic and toxic effects of urea which rather seems to act as a surrogate marker, reflecting behaviour of other uremic toxins with more serious impact [3]. Dialysis patients nutritional status is an essential parameter to follow since patients are losing proteins and amino acids in the course of the dialysis, which leads to muscle mass loss [4]. A frequently used marker for estimation of nutritional status in dialysis patients is urea nitrogen appearance in serum/urine/dialysate for the calculation of PNA (protein nitrogen appearance) and/or nPNA (normalized PNA) [5]. Another widely used small molecular weight marker for

* Corresponding author, jana@cb.ttu.ee

estimating kidney function is creatinine. Increase of serum creatinine is the result of uremic retention, but can also be a consequence of muscle breakdown [6]. A higher level of serum creatinine has been proved to be one independent significant predictor of long-term survival in incident dialysis patients [7].

Recent studies suggest that a high level of UA may play an important role in the development of hypertension, renal disease and cardiovascular events [8–12]. Hyperuricemia is defined by the concentration above 6.5 mg/dL (387 μmol/L) in women and 7 mg/dL (416 μmol/L) in men [13]. Studies of patients with chronic kidney disease (CKD) in relation to their serum UA concentration have given contradictory results; it has been found that: (a) higher UA level seems to be an independent threat factor for all cause and cardiovascular disease (CVD) mortality [14], (b) there is a J-shaped relationship between UA concentration and mortality [15,16], and (c) high serum UA content is related to lower risk for all cause and CVD mortality [17].

The purpose of this study was to examine if simultaneous monitoring of three small molecule uremic markers, urea, creatinine, and UA could be related to the survival of dialysis patients.

2. SUBJECTS AND METHODS

The studies were performed after authorization of the protocol by the Tallinn Medical Research Ethics Committee, Estonia, and Regional Ethical Review Board, Linköping, Sweden. All patients gave informed consent for participation. A summary of the studies and patients is presented in Table 1. All patients were on chronic thrice-weekly hemodialysis; dialysis dose was ensured according to the international guidelines [2].

The concentrations of urea, creatinine, and UA in the samples were determined in the Clinical Chemistry Laboratories (accuracy ±3–5%). In order to estimate the effect of the serum concentration of small uremic markers on survival, Kaplan–Meier analysis with log-rank test was performed, Statistica 9.0 (Statsoft Inc., US) was used. The mean follow-up period was 24 months (one to 45 months). Patients were divided into two groups according to their pre-dialysis urea, creatinine, or UA value and labelled "Urea/Creatinine/UA below/above average". Since concentration values were distributed normally (D'Agostino Pearson test $p > 0.05$), grouping was made according to the mean value of the group. Out of the 33 monitored patients, 22 died, three were transplanted, and eight survived. Logistic (logit) and Cox regression analyses were used for creating models for 3 years survival estimation among dialysis patients, Statistica 10.0 (Statsoft Inc., US) was used. Models were adjusted for age and used: (1) only urea based variables (concentration before the procedure, total removed amount (TR) and reduction ratio (RR)), (2) only UA based parameters (similar to urea), and (3) UA and urea parameters in combination. One set of models used values from blood and the second one from dialysate samples. Parameter values from the first session of the week were used. Although some parameters are linearly related, none of the parameters, included to the combined prediction models, had significant correlation with each other.

The total removed amount of a substance was calculated as follows:

$$TR = C_t W_t, \tag{1}$$

where C_t is the substance concentration in total dialysate collection tank and W_t is the weight of the dialysate collection tank (kg). It was assumed that 1 kg = 1 L of the dialysate.

The reduction ratio of substance was calculated as follows:

$$RR = \frac{C_0 - C_1}{C_0} 100\%, \tag{2}$$

where C_0 and C_1 are the substance concentrations at the beginning and at the end of dialysis, respectively.

Logit models for estimating the survival probability (z) were created in the following form:

$$z = \frac{\exp(a + b_1 x_1 + b_2 x_2 + b_3 x_3)}{1 + \exp(a + b_1 x_1 + b_2 x_2 + b_3 x_3)}, \tag{3}$$

where a is an intercept, b_i-s are slopes (regression coefficients), and x_i-s are the variables (e.g. concentration, TR and RR value).

Same logic and parameters were used in Cox regression analysis. The cumulative hazard H at a given time t was estimated as:

$$H(t) = H_0(t) \cdot \exp(b_1 x_1 + b_2 x_2 + b_3 x_3), \tag{4}$$

where $H_0(t)$ is the cumulative underlying hazard function.

The prognostic index (PI) was calculated as a combination of regression coefficients $(b_1 - b_3)$ and values of variables $(x_1 - x_3)$:

$$PI = b_1 x_1 + b_2 x_2 + b_3 x_3. \tag{5}$$

Three year survival probability for each patient was calculated as follows [18]:

$$S(3 \cdot 365) = \exp[-H_0(3 \cdot 365) \cdot \exp(PI)]. \tag{6}$$

The calculated probabilities were compared with real statuses for estimating prediction accuracy.

Table 1. Summary of the studies, participating patients, conditions, and samplings

	Survival analysis	Survival prediction models
Number of patients (M/F)	33(29/4)	18(14/4)
Tallinn/Linköping	0/33	10/8
Age ± SD	71 ± 12	69 ± 12
Months on dialysis, mean ± SD and min–max value	48 ± 55 5–301	30 ± 26 1–109
Kidney disease (rate of occurrence)	Atherosclerosis (5) Congenital malformations (1) Cystic kidney disease (6) Diabetic nephropathy (11) Glomerulonephritis (6) Hypertension (1) Interstitial nephritis (1) Drugs nephropathy (1) Unspecified CKD (1)	Atherosclerosis (2) Cystic kidney disease (2) Diabetic nephropathy (6) Glomerulonephritis (2) Goodpastures syndrome (1) Hypertension (2) Myeloma (1) Renal tuberculosis (1) Tubulointerstitial nephritis (1)
Dialysis access (N)	a/v fistula (27) Graft (1) Central dialysis catheter (5)	a/v fistula (11) Graft (4) Temporary catheter (3)
Dialyzer	FX80 FX800	FX8 FX10 FX80
Dialysis machine	Fresenius 5008	Fresenius 4008 Fresenius 5008
Blood flow, mL/min	250−390	250−390
Session's duration, min	180−270	180−270
Sampling time	Pre-dialysis serum UA/Urea level in the beginning of the study	Pre- and post-dialysis blood/dialysate samples. Sample from the dialysate collection tank
Mean ± SD and min–max value of serum Urea (Urea_B), mmol/L	20.3 ± 5.02 10.6–29.7	20.5 ± 5.25 13.9–32.6
Mean ± SD and min–max value of serum Creatinine, μmol/L	687 ± 160 463–1166	
Mean ± SD and min–max value of serum UA (UA_B), mg/dL (μmol/L)	5.75 (342) ± 1.10 (65.4) 3.36–8.64 (200−514)	6.53 (389) ± 1.04 (62.0) 4.71–9.18 (280−546)

Notes: Conversion factor for UA mg/dL to μmol/L, ×59.48.
Numerical values presented in the table were not statistically different between the groups ($p < 0.05$).

The performance of the models was evaluated by Receiver Operating Characteristic (ROC) curve analysis using MedCalc 12.7.2 (MedCalc Software, Belgium).

To determine the differences, the Student *t*-test was used and *p*-value lower than 0.05 was considered significant.

3. RESULTS

3.1. Dialysis patients' baseline urea, creatinine, and UA level and survival

Figure 1 shows Kaplan–Meier analysis, estimating the effect of dialysis patients' pre-dialysis serum urea (Urea_B), creatinine, and UA (UA_B) level in the beginning of the study on survival. Interestingly, survival was higher among patients having a higher urea concentration than the mean value of all the subjects (20.3 mmol/L). However, this trend was not significant (log-rank = 3.83; $p = 0.095$). The analysis showed that survival was independent on creatinine level (log-rank = –0.27; $p = 0.906$). Analysis showed a significant difference in survival between groups depending on whether the serum UA concentration in the beginning of the study was below or above the mean value of all the subjects (log-rank = 5.14; $p = 0.026$).

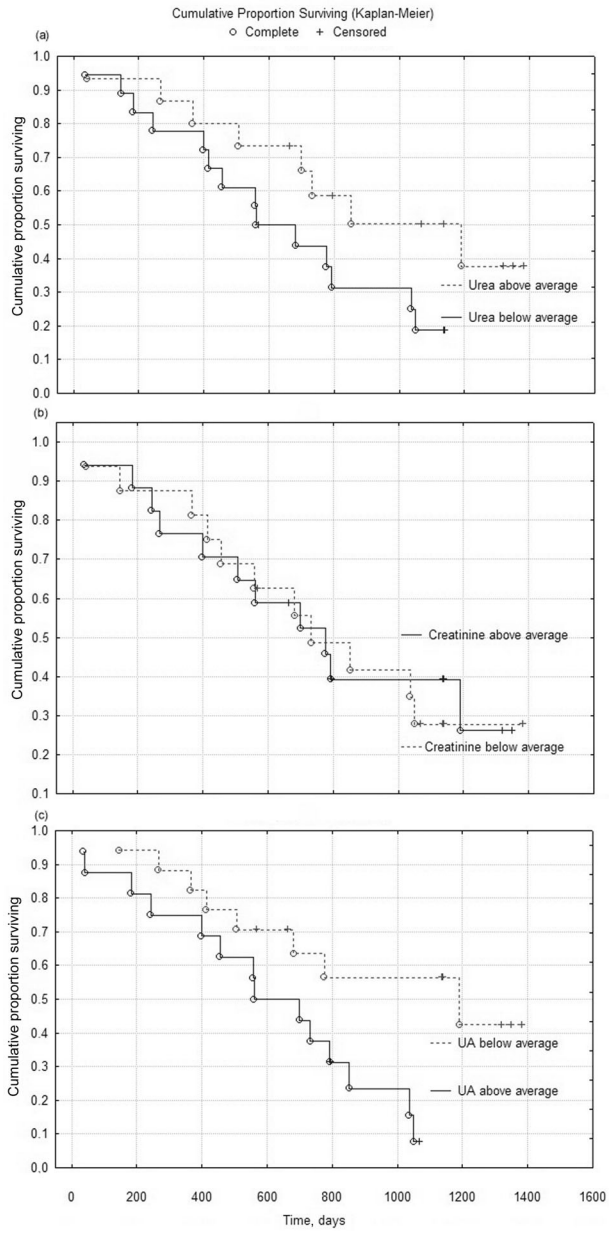


Fig. 1. Survival analysis of dialysis patients participating in the study. Grouping has been made according to the patient's serum pre-dialysis: (a) urea (log-rank = 3.83; $p = 0.095$), (b) creatinine (log-rank = –0.27; $p = 0.906$), and (c) UA (log-rank = 5.14; $p = 0.026$) level in the beginning of the study.

3.2. Survival prediction models

Summary of parameter values used in prediction models is shown in Table 2. In case of the studied group and parameters, logit models, especially combined ones, had higher specificity and sensitivity [$0.7 \le$ area under curve (AUC) ≤ 1.0] compared to Cox models ($0.65 \le$ AUC ≤ 0.98). However, Cox analysis assured that UA or combined models ensure better prediction than the urea based model. Table 3 presents the performance of the logit models.

1. **Model "UREA".** Parameters included: pre-dialysis urea concentration, reduction ratio (RR), and total removed (TR) amount.
2. **Model "UA".** The same parameters included as above but for UA.
3. **Model "UREA + UA".** Combination of UA and urea based parameters included: pre-dialysis UA, pre-dialysis urea, and RR of UA.

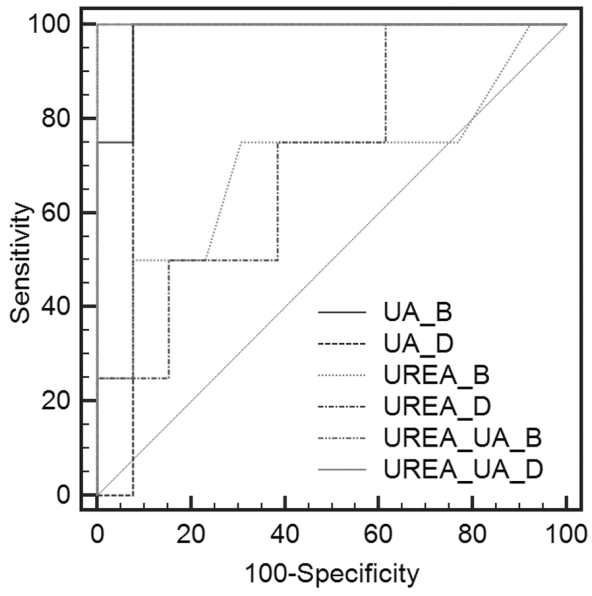
Table 2. Summary of mean ± SD values of parameters used in survival estimation models

	Survived after 3 years	Not survived after 3 years
N	5	13
UA_B, mg/dL	5.57 ± 0.65*	6.91 ± 0.93*
μmol/L	331 ± 38.9	410 ± 55.1
Urea_B, mmol/L	21.2 ± 7.60	20.3 ± 4.41
RR UA_B, %	69.1 ± 8.14	73.4 ± 8.53
RR Urea_B, %	70.7 ± 9.07	69.6 ± 6.28
UA_D, mg/dL	1.45 ± 0.35	1.76 ± 0.35
μmol/L	86.4 ± 21.1	104 ± 20.1
Urea_D, mmol/L	7.16 ± 2.92	7.09 ± 1.84
RR UA_D, %	68.7 ± 12.4	75.4 ± 11.5
RR Urea_D, %	65.4 ± 11.5	72.0 ± 9.61
TR UA, mmol	6.56 ± 2.31	7.72 ± 1.65
TR Urea, mmol	619 ± 291	583 ± 145

* Values were significantly different ($p < 0.05$).
Note: Conversion factor for UA mg/dL to μmol/L, ×59.48.

Table 3. Summary of logistic regression models for the estimation of three-year survival of dialysis patients. All models used altogether 3 serum/dialysate parameters of urea, UA or combination of both for prediction

	Blood samples			Dialysate samples		
	UREA	UA	UREA + UA	UREA	UA	UREA + UA
Accuracy, %	60.60	87.34	99.18	68.90	82.83	99.86
Chi-Square	0.27	14.24	20.96	4.20	8.65	18.55
p	0.9649	0.0026	0.0001	0.3798	0.0706	0.0010



	AUC	SE	95% CI[a]
UA_B	0.981	0.0272	0.772 to 1.000
UA_D	0.923	0.0769	0.688 to 0.996
UREA_B	0.702	0.199	0.436 to 0.894
UREA_D	0.712	0.156	0.446 to 0.900
UREA_UA_B	1	0	0.805 to 1.000
UREA_UA_D	1	0	0.805 to 1.000

AUC – area under ROC curve SE – standard error
CI – confidence interval
[a] Binomial exact

Fig. 2. ROC curves of the created models for estimating dialysis patient's 3 year survival. Models used 3 serum (B) or dialysate (D) parameters of urea, UA or combination of both for prediction.

One set of models used values from blood and the other from dialysate samples. Figure 2 shows Receiver Operating Characteristic (ROC) curve analysis of the created logit models.

4. DISCUSSION AND CONCLUSIONS

It has been suggested by different groups that there is a relation between patients serum UA level and CVD [8,19]. At the same time, classical markers for estimating kidney function are creatinine and urea [6], whereas serum urea concentration is considered to be one out of several indicators of nutritional status of dialysis patients [5]. This led us to examine the survival of the dialysis patients considering these three uremic markers. Patients were divided into two groups corresponding to their predialysis serum urea, creatinine and UA concentration in the beginning of the study. Higher survival rate among patients with higher serum urea concentration was observed, but this trend was not significant (Fig. 1a). The results could be explained by the assumption that higher urea level expresses better nutritional status of the patient. No difference in survival was determined in relation with creatinine levels (Fig. 1b). Survival test showed significant relationship between the serum UA concentration in the beginning of the study and mortality rate after 45 months from the start (Fig. 1c), demonstrating results similar to an earlier study by Madero et al. [14]. It was noted that patients with relatively high UA survive more likely if their serum urea level is also high. The latter finding might explain the described J-shaped relationship between UA levels and mortality [15,16]. Similarly to earlier studies [7], the current study indicates that solely following and fulfilling urea based hemodialysis dose quality parameters is not sufficient for predicting and achieving 3 year survival of the patients. The results evoke an idea of combining concentrations and removal of several molecules into a single model for predicting dialysis patient outcome. Since creatinine levels seemed not to be influential to dialysis patients' survival, prediction models used UA and urea based parameters.

To create models, which could indicate the survival probability of dialysis patients after three years, more thorough data (including RR and TR values) from Tallinn was added to Linköping's data. Figure 2 and Table 3 show that using combined logistic regression models lead to highly accurate results. It suggests that survival probability may be determined by a set of causal factors (pre-dialysis UA, pre-dialysis urea, and RR_UA).

The mean variable values used in models (Table 2) demonstrate that the parameters behave differently and in different directions within the groups. Distinctive feature for the survival group is the statistically lower UA value. However, the optimal model for survival estimation should also consider serum urea and removed UA values.

The main limitation of this study was the small study group. However, by our knowledge, this kind of parameter combining approach is unique and has a potential to improve the quality of dialysis, and hopefully also life expectancy of dialysis patients.

There were no considerable differences in the performance of the models whether the results from blood or dialysate samples were used. This indicates that a model, which uses dialysate values, could receive values from an optical monitoring system [20–23], enabling continuous monitoring of the dialysis efficacy and prognosis for patient's outcome. Optical monitoring of the urea based dialysis dose is considered as a valid and beneficial alternative to the existing methods [24].

In this study the effects of combining the small water soluble organic uremic retention solutes were investigated. The future goal is to test created models in a larger independent validation cohort and make adjustments if needed. Possible associations for other uremic

solutes with different molecule size, protein binding, and vascular calcification will be also explored.

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Dialüüsipatsientide elulemuse hindamine, kombineerides väikese molekulkaaluga ureemilisi markereid

Jana Holmar, Ivo Fridolin, Fredrik Uhlin, Anders Fernström ja Merike Luman

Dialüüsipatsientide elulemus on muret tekitavalt madal. Uuringu eesmärgiks oli kindlaks teha väikese molekulkaaluga ureemiliste jääkproduktide taseme mõju dialüüsipatsientide elulemusele. Samuti töötati välja mudelid, hindamaks patsientide elulemuse tõenäosust lähtuvalt kahe molekuli, uurea ja kusihappe kontsentratsioonist ning eemaldamisest. Uuringu käigus leiti, et kõrge kusihappe tase on statistiliselt oluliselt seotud patsientide kõrgema suremusega, kõrgel uurea tasemel on vastupidine mõju (ei olnud statistiliselt oluline) ja kreatiniini kontsentratsioon ei mõjuta elulemust. Leiti, et kusihappe ja uurea parameetreid kombineeriv mudel võimaldaks hinnata dialüüsipatsiendi kolme aasta elulemustõenäosust. Mudelite töökindlus ei sõltunud sellest, kas ennustamiseks kasutati vere- või kulunud dialüsaadiproovide väärtusi. Viimane võimaldaks prognoosimist ka optiliste seiremeetodite põhjal. Uuringus osalenud patsientide arv oli väike ja tulevikus peab mudeleid kontrollima suuremas, sõltumatus grupis.