Mutual interaction of clinical factors and specific microRNAs to predict mild cognitive impairment in patients receiving dialysis

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Abstract

Background To examine mutual interaction of various clinical factors for cognitive impairment in patients receiving dialysis.

Methods A total 48 dialysis patients with subjective memory complaints in outpatient clinic were recruited from 2015 to 2017. Demographics, circulating uremic toxin concentrations, miRNA concentrations and nerve injury protein concentrations were collected and measured. Clinical dementia rating (CDR) scores was used to stratify the functional scores of the patients. Receiver operating characteristic(ROC) analysis was used to evaluate diagnostic test performance for predicting dichotomous results, cumulative ROC analysis to examine the combined contribution of clinical factors.

Results CDR scale 0 included 15 patients (mean age, 59.1 years; 5 men and 10 women); CDR > 0.5 included 33 patients (mean age, 64.0 years; 18 men and 15 women). On cumulative ROC analysis, the major predictors of mild cognitive impairment were hemoglobin, age, sex, homocysteine, neuron-specific enolase and miR-486. The cumulative AUC on combining hemoglobin, age, and miR-486 was the highest (0.897, 95% confidence interval 0.806–0.988). Two dichotomized variables reached 81.82% sensitivity and 86.67% specificity, with the likelihood ratio for positive and negative results being 6.14 and 0.21, respectively.

Conclusion Hemoglobin, age, and miR-486 exerts combined effects on mild cognitive impairment in patients receiving dialysis.

Background

Cognitive impairment (CI) has a high prevalence in chronic kidney disease (CKD), especially in elderly patients[1–3]. The clinical picture consists of cognitive slowing and executive, memory, and language deficits. The contributing factors include cerebral white matter disease[4, 5], silent brain infarcts[6, 7], demographic factors[8–11], vascular risk factors[12], uremic toxins[13], secondary hyperparathyroidism[14], and dialysis disequilibrium[15].

The CI observed in CKD influences not only daily life and ability to work, but also results in longer hospitalization and higher risk for mortality[13, 16, 17]. In the patients receiving hemodialysis exhibiting CI, the average time to death was 1.09 years, and hazard ratio (HR) for death was 1.87, which was higher than that observed in patients receiving hemodialysis with cardiac disease (HR, 1.28) or stroke (HR, 1.20). [13]

Although various factors may contribute to CI in patients with CKD, these factors are generally treated individually, without considering possible mutual interactions. Recently, receiver operating characteristic (ROC) analysis is being widely used to evaluate diagnostic test performance for predicting dichotomous results, by comparing sensitivity and specificity[18, 19]. Further, ROC analysis has been improved by simultaneously considering multiple factors, termed cumulative ROC analysis.

In the current study, we aimed to use ROC analysis to examine the contribution between demographics (age and sex), routine biochemistry variables, uremic toxins, and miRNAs for CI prediction in patients receiving dialysis. We also aimed to identify high risk factors through cumulative ROC analysis.

Methods

Participants

The study period was from 2015 to 2017. Patients who received regular outpatient hemodialysis/hemodiafiltration (thrice weekly) and peritoneal dialysis at Kaohsiung Chang Gung Memorial Hospital in Taiwan were enrolled. Inclusion criteria were: (1) age $\geq$ 18 years; (2) subjective memory complaints; (3) ability to provide basic interview, meaning no aphasia. Exclusion criteria included: (1) history of cerebral stroke or brain injury by non-medical causes; (2) drug-usage history, which may influence cognitive function, including chemotherapy; (3) other comorbidities, which may affect cerebral function, e.g., liver cirrhosis, cancer, and psychiatric diseases; (4) malnutrition, serum albumin level <3.5 g/dL; (5) alcoholism; (6) pregnancy; (7) hospitalization within 3 months of enrollment; (8) absence of caregiver for providing medical history. All of the participants underwent hemodialysis with dialyzers of surface area $\geq$ 2.0 m^2 and bicarbonate-based dialysate. The method of replacement solution supply in hemodiafiltration was predilution. Peritoneal dialysis was performed with varying concentrations of glucose-based PD solutions (1.36%, 2.27%, and 3.86%; Baxter Healthcare SA, Singapore), depending on the prescription of their respective nephrologists.

Laboratory measurement

Blood sampling was performed in the mid-week (Wednesday/Thursday) with fasting status for participants who had received hemodialysis/hemodiafiltration. Participants receiving peritoneal dialysis underwent blood examination in the first week of the month. Blood test for uremic toxins included: (1) small water-soluble solutes, such as blood urea nitrogen (BUN), creatinine(Cr), calcium(Ca), phosphorus(P), asymmetric dimethylarginine (ADMA), 8-hydroxy-2-deoxyguanosine (8-OHdG); (2) protein-bound solutes, such as p-cresyl sulfate (PCS), indoxyl sulfate (IS), homocysteine; (3) medium-sized molecules: interleukin 1-β (IL-1β), interleukin 6 (IL-6), interleukin 18 (IL-18), tumor necrosis factor-α (TNF-α), intact parathyroid hormone (iPTH), β2 microglobulin. Blood samples for BUN, Cr, Ca, P and glutamate oxaloacetate transaminase (GOT) were analyzed using commercial kits and an autoanalyzer (Hitachi 7600-210, Hitachi Ltd., Tokyo, Japan). The PCS and IS were quantified using HPLC (Agilent 1100 series, USA). iPTH was measured by chemiluminescent immunoassay (Siemens Healthcare Diagnostics Inc., USA). Measurements of ADMA, 8-OHdG, IL-1β, IL-6, IL-18, and TNF-α were performed using ELISA. β2 microglobulin level was measured using a turbidimetry method (Spaplus, The Binding site Group Ltd, UK).

Nerve-injury proteins

We measured 3 nerve injury related proteins in the blood using ELISA: neuron-specific enolase (NSE); heat shock protein (HSP) 70 and S100B.

Measurement of serum miRNAs levels

We selected candidate miRNAs from peripheral mononuclear cells using next generation sequencing (NGS). The candidate miRNAs were selected if their blood levels were higher than 1.5-fold in patients receiving dialysis compared to healthy controls. Thus, 4 miRNAs were identified: miR-134, miR-182, miR-451, and miR-486.

Method of NGS

The collected RNA samples were first subject to quality examination with Bioanalyzer 2100 (Agilent) facility. The RNA samples with RIN (RNA integrity number, determined with Bioanalyzer 2100) value ≧ 8.0 were prepared with TruSeq Small RNA Preparation protocol (Illumina), followed by sequencing with a V3 150-cycle sequencing reagent on the MiSeq facility (Illumina) to generate 51-nt single-end reads. The generated NGS data were analyzed with miRSeq tool kit file:///D:/Documents/My_Papers/FINISHED/51_ADHD_miRNA_Diagnosis panel/SUBMISSION/Manuscript_R1.doc - _ENREF_1#_ENREF_1[20] with default parameters to quantify the expressions of human miRNAs (miRBase 20).

Quantitative PCR for miRNAs

For quantitative PCR of the miRNAs, 10 ng of total RNA was converted into cDNA using the TaqMan MicroRNA Reverse Transcription kit (Thermo Fisher Scientific Applied Biosystems, Foster City, USA). The expression of mature miR-134, miR-182, miR-451, miR-486, and U6 (internal control) was quantified using the commercially available TaqMan Universal Master Mix No Amp UNG (Applied Biosystems, Foster City, USA) in a 7500 Real-Time PCR System. The qRT-PCR was performed at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Expression levels of the miRNAs were normalized to that of the internal control, U6, by using the equation log (2-ΔCt), where ΔCt = Ct target - U6.

Mini-Mental State Examination

General cognitive function was assessed using the Mini-Mental State Examination (MMSE) [21]. We used clinical dementia rating (CDR) scores to stratify the functional scores of the patients[22]. The CDR rated the participant's impairments in 6 categories – memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care –on a 5-point scale (0, 0.5, 1, 2, and 3). Summarizing the impairment ratings, all of the participants were assigned a rating score of CDR-0 indicating no dementia, and 0.5, 1, 2, 3 indicating questionable, mild, moderate, and severe dementia, respectively. The necessary information was obtained through interview of the patient and reliable informant (eg. family member) by one qualified reviewer based on CDR assessment protocol.

Statistical analyses

The distribution of variables between the groups was summarized in terms of mean (standard deviation), median (interquartile range), or frequency (percentage). The difference between the groups was

estimated using the independent two-sample t-test or χ^2 test, as appropriate. In addition, the effect size of continuous variables between groups is estimated using Cohen's d from t-test. The dichotomous result of each variable was determined by individual ROC analysis. Higher individual area under curve (AUC)s represent better prediction performance for high CDR scores. The variables were then ranked by individual AUCs estimated from individual ROC analysis.

A cumulative ROC analysis was performed to detect the combined effects of the measurements used to predict a high CDR score. Positive changes in the cumulative AUCs were tracked along with the cumulated variables, until the addition of other variables no longer increased the cumulative AUC. In cumulated ROC analysis, the likelihood ratio was used to assess high CDR scores in subjects with different cumulative scores. The LR+ (sensitivity/[1 - specificity]) represents the ratio of the probability of a positive test for the subjects with high CDR scores to that of a positive test for the subjects with low CDR scores. Therefore, the LR– ([1 - sensitivity]/specificity) represents the ratio of the probability of a negative test for the subjects with high CDR scores to that of a negative test for the subjects with low CDR scores. The 95% confidence interval for sensitivity, specificity, and likelihood ratios were computed.

Variables which contributed to positive changes for the highest cumulative AUC were selected for further analysis. The cumulative risk score for each subject was obtained by adding the risk score (1 or 0) of selected variables which contributed to the high CDR score. For each subject, the cumulative risk score represented the total number of risk factors. For instance, a score of 5 was interpreted as the presence of 5 risk factors which were most relevant to a high CDR score. In general, the limited sample size was provided unusable results (eg. wide confidence interval) and also generate an overfit model in regression analysis. Considering the above-mentioned limitations, the logistic regression was conducted and included only the most contribute factors for mild cognitive impairment in patients receiving dialysis derived from cumulative ROC results. A *P* value less than 0.05 is considered statistically significant. All statistical analyses were performed using STATA version 11.0.

Results

Baseline characteristics

114 potentially eligible dialysis patients were screening. Finally, a total 48 patients receiving dialysis were enrolled for analysis(Figure 1). Thirty-three patients received hemodialysis therapy, 7 patients with peritoneal dialysis and 8 with hemodiafiltration therapy. Dialysis vintage was 119 $\pm$78 months. CDR scale 0 included 15 patients (mean age, 59.1 years; 5 men and 10 women); CDR $\geq$ 0.5 included 33 patients (mean age, 64.0 years; 18 men and 15 women). The distribution of CDR in patients with different dialysis modality was shown in supplementary Table 1. There were no significant differences in the blood levels of biochemical variables, small water-soluble solutes, protein-bound solutes, medium-sized molecules, and molecular markers of nerve-injury, except that the BUN level was higher in the patients with CDR 0 (t-test, p=0.009; Cohen's d 0.88) and the hemoglobin (Hb) level was higher in the patients with CDR $\geq$ 0.5 (t-

test, p=0.010; Cohen's d -0.97). The candidate miRNA analysis did not show significant difference in the blood levels of miR-134, miR-182, miR-451, and miR-486 (Table 1).

Area under curve (AUC) for individual clinical and laboratory factors for CI prediction in patients receiving dialysis

Table 2 presents the dichotomous results for the various single demographic and laboratory factors listed in Table 1. None of the factors showed acceptable performance (AUC $\geq$ 0.7), except Hb (AUC = 0.792) and age (AUC = 0.708).

Cumulative ROC analyses for CI prediction in patients receiving dialysis

Table 3 shows the cumulative top-ranked clinical factors for predicting CI in patients receiving dialysis. The cumulative AUC for the combination of cumulative top-ranked clinical factors was calculated by using the AUC for different combinations of factors, where the factors were added individually, in the descending order of individual AUCs (Table 2). The highest score was obtained for a combination of 3 top-ranked clinical factors, including Hb, age, and miR-486 (cumulative AUC = 0.897, 95% confidence interval [CI] 0.806–0.988). The second combination involved 4 top-ranked clinical factors: Hb, age, miR-486, and sex (cumulative AUC = 0.874, 95% CI 0.768–0.981). Combinations of other top-ranked clinical factors showed a descending order of cumulative AUCs. Accordingly, Hb, age, sex, miR-486, homocysteine, and NSE were the predominant factors for predicting CI in patients receiving dialysis. Further, we calculated sensitivity and specificity for these dichotomized variables. Two out of 3 top-ranked variables (Hb, age, miR-486) showed a highest Youden's index (68.49%) and correctly classified percentage (83.33%) than others (Table 4). The sensitivity was 81.82% and the specificity was 86.67%, classified with a likelihood ratio for positive (LR+) and negative (LR-) results being 6.14, 0.21, respectively. The relationship between cumulated risk score and selected variables (Hb, age, miR-486) were summarized in Table 5, by interpreting the proportion of patients in each cumulated risk score strata. The patients with cumulated risk score 1 have highest proportion in miR-486 $\geq$ 32.68 (66.7%).

The information of distribution of MMSE in different CDR groups was shown in supplementary Table 2. The results of cut-off point of cumulated risk score identified by ROC analysis for MMSE category derived from CDR dichotomous stratification was shown in supplementary Table 3. Two out of 3 top-ranked variables (Hb, age, miR-486) showed a highest Youden's index (24.17%) and correctly classified percentage (62.50%) than others. The sensitivity was 72% and the specificity was 52.17%, classified with a likelihood ratio for positive (LR+) and negative (LR-) results being 1.51, 0.54, respectively. This relationship was shown in supplementary Figure 1. This result indicated CDR stratification was superior to MMSE stratification in examining association of clinical factors with cognitive impairment in our participants.

The cumulative ROC results indicated Hb, age, and miR-486 exerts major contribution, and hence, we included these variables for logistic regression analysis (Table 6). The univariate analysis indicates the association for each of the included variables for mild cognitive impairment. Whereas, the multivariate

analysis considered the association simultaneously. Both univariate and multivariate analysis indicate the Hb [Univariate: OR (95% CI) = 2.29 (1.13 - 4.64), *P* = 0.022; Multivariate: OR (95% CI) = 2.74 (1.13 - 6.67), *P* = 0.026] and miR-486 [Univariate: 4.24 (1.14 - 15.79), *P* = 0.031; Multivariate: OR (95% CI) =7.54 (1.47 - 38.6), *P* = 0.015] were significantly associated with mild cognitive impairment. Afterward, the 2-order interaction analysis including Hb*age [OR (95% CI) =1.01 (1.001 - 1.01), *P* = 0.019], Hb*miR-486 [OR (95% CI) =1.17 (1.03 - 1.32), *P* = 0.016] and age*miR-486 [OR (95% CI) =1.03 (1.004 - 1.05), *P* = 0.019] were given. The results indicate all of the three combinations in 2-order interaction level were significantly associated with mild cognitive impairment in dialysis patients. Unfortunately, the 3-order interaction analysis were omitted due to the limited sample size and current regression algorithm. Overall, the 2-order interaction results were consistent with the cumulative ROC findings, which indicate hemoglobin, age, and miR-486 were associated for mild cognitive impairment in patients receiving dialysis under certain interaction effects.

Discussion

One of the risk factors for CI is CKD. The potential causes involve vascular and neurodegenerative mechanisms[23]. The implicated factors range from traditional/non-traditional ones to direct neuronal toxicity due to uremic toxins[23]. However, the combined effect of these factors on CI in CKD remains unknown. Phenotype presentation of a disease is the consequence of combined effect of various molecules and signaling pathways in the organism. Therefore, we applied ROC analyses to examine the combined effect of plausible clinical factors for CI prediction in patients receiving dialysis. Based on baseline comparison, we did not find significant difference in the proposed factors between CI and non-CI participants. We also selected four candidate miRNAs using next generation sequencing, miR-134, miR-182, miR-451, and miR-486 to compare between the CI and non-CI participants. The individual levels of these miRNAs did not show significant differences between the two cohorts. When we applied individual ROC analysis, Hb and age were the prominent factors, reaching AUC > 0.7. Further, we applied cumulative ROC analyses to examine the combined effect of the proposed factors. The combination of Hb, age, and miR-486 showed the best cumulative AUC for CI prediction in patients receiving dialysis. The other important combined factors included sex, homocysteine, and NSE (a nerve-injury protein). Thus, we propose that a combined effect of clinical factors contributing to mild CI in patients receiving dialysis.

It has been reported that Hb shows a U-shaped association with cross-sectional cognitive function[24, 25]. The plausible underlying mechanisms are inadequate cerebral oxygenation leading to impaired cerebral perfusion and cerebral function in low Hb concentrations[26]. In contrast, high Hb concentrations may represent hyperviscosity, hypovolemia, polycythemia vera, and pulmonary disease. These scenarios may lead to cerebral hypoxia and cognitive impairment[24, 27]. However, there is still no evidence to show that optimal Hb concentrations prevent CI in CKD. In the present study, we found that Hb showed the highest AUC on individual ROC analysis. Moreover, a cumulated ROC analysis exhibited an acceptable AUC for CI prediction in patients receiving dialysis. Based on our findings, we propose that future studies on an optimal Hb concentration for stratified age groups of subjects are necessary for prevention or treatment of CI.

Aging is a natural process in organisms. Complex sophisticated coordinated mechanisms among tissues and organs are involved in the aging process. Commonly, aging is characterized by the progressive decline in functions of the tissues and organs. Eventually, aging contributes to the risk of disease occurrence. In the past decades, studies have investigated the molecular mechanisms of aging in different tissues[28-31]. Aging is well-recognized as the greatest risk factor for the onset of age-related neurodegenerative diseases. Age-related alterations in the brain include cell adhesion molecules, neuronal activity, and neurotransmitter and neuromodulator action[29]. Our findings echo the aforementioned reports that aging contributes to CI in patients receiving dialysis. Nevertheless, this study is not sufficient to test our hypothesis for the plausible mechanism of age-related CI in patients receiving dialysis. An advanced study is warranted to explore the aging mechanism underlying the onset of CI in patients receiving dialysis.

Sex showed the fourth-highest AUC in our study. The cumulative AUC of Hb, age, sex, and miRNA-486 was 0.874. This finding indicates that sex plays an important role in CI of patients in Taiwan receiving dialysis. A nationwide survey in Taiwan showed that women had a higher prevalence than men for overall dementia and mild CI [32]. The exact explanation was not provided by the authors. In our study, we did not include economic status, educational levels, and comorbidities for comparison between men and women. Therefore, we can only conclude that sex contributes to CI in patients receiving dialysis. Further studies are needed to address the plausible mechanisms underlying the above observations.

We analyzed the contribution of several uremic toxins to CI in our study. Among them, homocysteine showed the highest AUC on individual ROC analysis. Homocysteine is a protein-bound solute, and is commonly elevated in patients with CKD. On conversion to homocysteic acid, homocysteine can activate N-methyl-D-aspartate receptor, thus, leading to a direct neurotoxic effect[33]. Homocysteine has been shown to be associated with faster rate of cognitive decline in a 6-year follow-up study on elderly subjects[34]. Our study found homocysteine was one of cumulated top-ranked factors for prediction of mild CI in patients receiving dialysis. The cumulative AUC was 0.835 when 5 factors (Hb, age, miR-486, sex, and homocysteine) were combined. However, the role of homocysteine in causing CI in patients receiving dialysis must be validated by a large-scale population study.

Circulating miRNAs have been reported to be biomarkers of mild CI. Two sets of miRNA pairs, miR-132 and miR-134 families, were shown to differentiate patients with mild CI from age-matched controls[35]. Next generation sequencing for miRNA expression profiling is becoming a common technology for various diseases, including those affecting the kidney and brain[36, 37]. We used this technology to profile miRNAs in patients receiving dialysis, and identified 4 candidate miRNAs: miR-134, miR-182, miR-451, and miR-486. In our study, the plasma levels of individual miRNAs were not statistically different between patients with and without CI. Using cumulative ROC analyses, we found that the combination of Hb, age, and miR-486 showed an acceptable cumulative AUC (0.897). It seems that miR-486 plays a crucial regulatory role in CI in patients receiving dialysis. One of the miR-486 target genes, *GABRB3*, encodes gamma-aminobutyric acid receptor subunit beta-3 (GABRB3), which is a member of the ligand-gated ion channel family[38]. Gamma-aminobutyric acid is the major inhibitory neurotransmitter of the

nervous system. The missense mutation of this gene may be associated with several nervous diseases, including epilepsy and autism[39]. However, the regulatory roles of miRNAs are complex. Individual miRNAs can target several mRNAs, and an mRNA can be targeted by more than one miRNA. This might, at least in part, explain why we could not find a role of the CI-related miR-134[35] in the patients receiving dialysis. The identified miRNAs for mild CI in these patients were different from those previously reported in subjects without CKD[35]. Currently, the evidence for differences in CI-related miRNA profiles between CKD and non-CKD cohorts is still lacking. Further studies on disease-specific miRNAs are required to clarify this issue.

A total of 152 uremic toxins have been detected, and these molecules have been shown to exert various negative effects, such as anorexia, cardiac failure, anemia, immune dysfunction, malnutrition, inflammation, and skin atrophy[23, 40]. Uremic toxins have also been suspected to have a causal relationship with CI in CKD[41]. However, the impact and mechanism of action of each uremic toxin on cognition and cerebral nervous system in uremic state remains unknown. In our study, homocysteine and β2 microglobulin exhibited higher AUC than other uremic toxins. Guanidine compounds were found in the brain regions involved in cognition[42]. These compounds reportedly indirectly elevate serum homocysteine in cognitive disorders[43]. The definitive mechanisms of action of uremic toxins for the onset of CI in CKD require further investigation.

In our study, we measured blood levels of nerve-injury related proteins, such as NSE, HSP 70, and S100B; NSE was one of the top-ranked variables on cumulative ROC analyses. It is a glycolytic isoenzyme expressed in the central and peripheral neurons and neuroendocrine cells. In rats, NSE immunofluorescence signal decreased in the affected neurons 2–10 days following axonal injury. There is accumulating evidence that the level of NSE in neurons serves as a marker of axon injury, regeneration, and target reinnervation[44-46]. Accordingly, we propose that NSE might be involved in the onset of CI in patients receiving dialysis.

Limitations of the study

The present study is subject to several limitations. Firstly, our study did not include follow-up interval, and the cumulative ROC approach was limited to cross-sectional clinical data. This may ignore all possible combinations of complex interactions when the effect of time is considered. Secondly, our study did not include other possible factors affecting CI in patients receiving dialysis, such as economic and social status, comorbidities, and dialysis protocols. Thirdly, we did our best to examine the implicated uremic toxin levels in our patients. However, there are several uremic toxins that we did not examine. Therefore, their contributions to CI in these patients was not be investigated. Fourthly, the sample size was relative small, and all the participants were Taiwanese. The possible effect of ethnicity cannot be ruled out in our study. Finally, cognitive impairment was determined solely by a psychometric classification and not a clinical diagnosis. Although the CDR is considered to be a reliable indicator of cognitive status, the lack of a more thorough cognitive characterization of the sample should be pointed out. Despite the above limitations, the strength of our study is in being the first investigation on a combination of specific clinical

factors predicting mild CI in dialysis patients. This approach not only provides an alternative insight into the plausible mechanisms for CI but also a method for CI prediction in such patients.

Conclusion

The cumulative ROC analysis provides better AUC performance than individual ROC analysis for identifying factors for prediction of mild CI in patients receiving dialysis. Our proposed scoring system identified 6 clinical factors which displayed a high degree of combined effect for mild CI in patients receiving dialysis.

Abbreviations

CI= cognitive impairment; CKD= chronic kidney disease; HR=hazard ratio; ROC= receiver operating characteristic; AUC= area under curve; CDR=clinical dementia rating; NSE=neuron-specific enolase; HSP=heat shock protein; BUN=blood urea nitrogen; Cr=creatinine; Ca=calcium; P=phosphorus; ADMA=asymmetric dimethylarginine; 8-OHdG=8-hydroxy-2-deoxyguanosine; PCS=p-cresyl sulfate; IS=indoxyl sulfate; IL= interleukin; TNF-α=tumor necrosis factor-α; iPTH=intact parathyroid hormone; GOT=glutamate oxaloacetate transaminase; Hb=hemoglobin; CI= confidence interval.

Declarations

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Authors' contributions

JBC design the study and drafted the manuscript. CCC design neurological examinations and interpretation of data, LCL, WCL, CNL design uremic toxin examinations and interpretation of data, SCL design NGS examination, SHM performed statistical analysis. CHY critically reviewed the statistical data and revised it critically for important intellectual content.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author, JBC, upon reasonable request.

Ethics approval and consent to participants

The study protocol was approved by the Committee on Human Research at Kaohsiung Chang Gung Memorial Hospital (number: 104-2572B). Informed consent obtained from participants was written. The study was conducted in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable

Competing interests

No potential conflict of interest was reported by the author(s).

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Tables

Table 1. Baseline characteristics of study participants. (N=48)

Variables	CDR=0 (n=15)		CDR≧0.5 (n=33)		*P*	Cohen's *d*
	Mean	SD	Mean	SD		
Age,years	59.1	±8.1	64.0	±9.5	0.092	-0.50
Gender (men, %)	5	33.33%	18	54.55%	0.173	
Education level					0.786	
No	0	0.00%	2	6.06%		
Primary school	5	33.33%	6	18.18%		
Elementary school	2	13.33%	4	12.12%		
High school	5	33.33%	11	33.33%		
Bachelor	2	13.33%	4	12.12%		
Unknown	1	6.67%	6	18.18%		
Laboratory measurement						
Kt/V	1.83	±0.39	1.82	±0.41	0.968	-0.001
Hb(g/dL)	9.92	±0.9	10.83	±1.15	0.010	-0.97
Albumin(g/dL)	3.80	±0.24	3.84	±0.36	0.685	-0.14
GOT(U/L) (median, interquartile range)	19	14-25	18.5	15-27.5	0.404	-0.26
Small water-soluble solutes						
ADMA(umol/L) (median, interquartile range)	3.28	0.9-5.74	3.28	0.63-5.95	0.511	-0.23
8OHDG(ng/ml)	27.48	±0.67	27.07	±0.69	0.058	0.52
BUN(mg/dL)	75.00	±23.38	59.36	±15.9	0.009	0.88
Cr(mg/dL)	10.72	±4.29	9.31	±2.4	0.149	0.45
Ca(mg/dL)	9.51	±0.68	9.22	±1.77	0.492	-0.08
P(mg/dL)	5.76	±2.21	7.53	±15.39	0.661	0.50
K(mEq/L)	4.47	±0.84	5.86	±8.86	0.551	-0.21
Protein-bound solutes						
PCS(ug/ml)	25.14	±16.81	27.00	±21.18	0.766	-0.22
IS(ug/ml)	46.05	±20.94	38.85	±18.83	0.241	0.28
Homocysteine(umol/ml)	26.82	±7.9	29.70	±10.15	0.351	-0.29
Middle molecules						
IL-1β(pg/ml) (median, interquartile range)	0.76	0.65-0.95	0.66	0.65-0.85	0.728	-0.13
IL-6(pg/ml) (median, interquartile range)	6.47	2.42-10.92	4.18	2.8-5.34	0.275	0.31
IL-18(ng/ml) (median, interquartile range)	111.99	90.13-166.47	108.64	90.78-140.18	0.122	0.50
TNF-α(pg/ml)	35.39	±10.98	34.88	±12.34	0.893	-0.03
iPTH (pg/dL)	260.7	108.7-715.8	190.85	121.55-507.4	0.392	0.18
Beta-2-microglobulin(ug/L) (median, interquartile range)	27700	20740-31619.5	25933.95	20695.75-31985.15	0.835	0.13
Molecular markers of nerve injury						
NSE(ng/ml) (median, interquartile range)	1556.04	936.89-2952.33	2418.23	1084.04-3520.94	0.896	-0.04
HSP 70(ng/ml) (median, interquartile range)	0.14	0.08-0.16	0.13	0.11-0.14	0.294	-0.38
S100B(pg/ml) (median, interquartile range)	83.58	57.05-142.02	83.58	25.26-157.98	0.892	-0.13
MicroRNA						
miR-134 (median, interquartile range)	0.53	0.33-1.77	0.51	0.15-2.48	0.563	-0.17
miR-182 (median, interquartile range)	0.09	0.03-0.36	0.06	0.04-0.23	0.970	-0.04

miR-451 (median, interquartile range)	4.92	0.36-10.69	1.9	0.49-10.04	0.284	-0.32
miR-486 (median, interquartile range)	32.38	22.18-188.2	111.14	33.96-269.09	0.643	-0.07

P-value was estimated using independent two-sample t-test or χ^2 test appropriately.

Cohen's *d* effect size corrected for uneven groups from t-test.

Abbreviations: Hb, hemoglobin; GOT, glutamate oxaloacetate transaminase; ADMA, asymmetric dimethylarginine; 8OHDG, 8-hydroxy-2-deoxyguanosine; BUN, blood urea nitrogen, Cr, creatinine; Ca, calcium; P, phosphate; K, potassium; PCS, p-cresyl sulfate; IS, indoxyl sulfate; IL, interleukin; TNF-α, tumor necrosis factor-α; iPTH, intact parathyroid hormone; NSE, neuron-specific enolase; HSP, heat shock protein.

Table 2. Individual ROC analysis of clinical measurements for CDR status.

Variable	AUC	Best Cutoff value	Sensitivity	Specificity	Correctly Classified
Age	0.708	63	69.70%	66.67%	68.75%
Gender	0.606	Male	54.55%	66.67%	58.33%
Education level	0.517	Above Elementary school	70.37%	35.71%	58.54%
Laboratory measurement					
Kt/V	0.470	1.3	100.00%	13.33%	72.34%
Hb	0.792	10.7	66.67%	93.33%	75.00%
Albumin	0.503	4.08	28.13%	86.67%	46.81%
Small water-soluble solutes					
ADMA	0.476	1.85	60.61%	46.67%	56.25%
8OHDG	0.339	25.6	100.00%	0.00%	68.75%
BUN	0.297	31	96.97%	0.00%	66.67%
Cr	0.409	11.2	33.33%	73.33%	45.83%
Ca	0.487	10.3	24.24%	93.33%	45.83%
P	0.406	3.8	84.85%	20.00%	64.58%
Protein-bound solutes					
PCS	0.497	53.8	15.15%	100.00%	41.67%
IS	0.398	21.1	93.94%	13.33%	68.75%
Homocysteine	0.571	27.97	65.63%	57.14%	63.04%
Middle molecules					
IL-1β	0.405	16.41	3.03%	100.00%	33.33%
IL-6	0.393	2.49	81.82%	26.67%	64.58%
IL-18	0.439	99.6	60.61%	46.67%	56.25%
TNF-α	0.481	53.5	9.09%	100.00%	37.50%
iPTH	0.464	54.4	87.50%	20.00%	65.96%
Beta-2-microglobulin	0.504	29040	40.63%	73.33%	51.06%
Molecular markers of nerve injury					
NSE	0.565	2418.23	51.52%	73.33%	58.33%
HSP 70	0.477	0.06	96.97%	13.33%	70.83%
S100B	0.477	227.27	18.18%	93.33%	41.67%
MicroRNA					
miR-134	0.501	1.22	40.63%	73.33%	51.06%
miR-182	0.483	0.02	93.75%	14.29%	69.57%
miR-451	0.503	0.93	69.70%	46.67%	62.50%
miR-486	0.614	32.68	78.79%	53.33%	70.83%

Table 3. Cumulated top-ranked predictors by using ROC analysis.

Cumulated top-ranked variables[*1]	Variable	Cumulative AUC	Standard error	95% Confidence Interval
2	Hb and Age	0.837	0.065	0.71-0.965
3	Above plus miR-486	0.897	0.047	0.806-0.988
4	Above plus Gender	0.874	0.054	0.768-0.981
5	Above plus Homocysteine	0.835	0.070	0.698-0.971
6	Above plus NSE	0.848	0.063	0.725-0.971
7	Above plus Education level	0.828	0.064	0.702-0.954
8	Above plus Beta-2-microglobulin	0.824	0.065	0.697-0.951
9	Above plus Albumin	0.827	0.068	0.694-0.959
10	Above plus miR-451	0.798	0.072	0.657-0.939
11	Above plus miR-134	0.794	0.076	0.644-0.943
12	Above plus PCS	0.799	0.075	0.652-0.946
13	Above plus Ca	0.819	0.070	0.682-0.955
14	Above plus miR-182	0.800	0.073	0.658-0.943
15	Above plus TNF-α	0.806	0.071	0.666-0.945
16	Above plus HSP 70	0.799	0.074	0.655-0.943
17	Above plus S100B	0.800	0.075	0.653-0.947
18	Above plus ADMA	0.815	0.073	0.671-0.958
19	Above plus Kt/V	0.823	0.073	0.681-0.965
20	Above plus iPTH	0.833	0.073	0.69-0.976
21	Above plus IL-18	0.835	0.068	0.701-0.968
22	Above plus Cr	0.810	0.074	0.664-0.955
23	Above plus P	0.812	0.075	0.666-0.958
24	Above plus IL-1β	0.812	0.075	0.666-0.958
25	Above plus IS	0.804	0.075	0.657-0.951
26	Above plus IL-6	0.808	0.074	0.662-0.954
27	Above plus 8OHDG	0.808	0.074	0.662-0.954
28	Above plus BUN	0.804	0.075	0.658-0.951

[*]The sequential of variable was depends on the value of individual AUC.

Table 4. Cut-off point of cumulated risk score identified by ROC analysis.

Number of dichotomized variables*	Sensitivity (95% CI)	Specificity (95% CI)	Youden's Index	Correctly classified	LR+ (95% CI)	LR- (95% CI)
1	100% (89.4% - 100%)	26.67% (7.79% - 55.1%)	26.67%	77.08%	1.36 (1.01 - 1.85)	-
2	81.82% (64.5% - 93%)	86.67% (59.5% - 98.3%)	68.49%	83.33%	6.14 (1.67 - 22.5)	0.21 (0.1 - 0.44)
3	27.27% (13.3% - 45.5%)	100% (78.2% - 100%)	27.27%	50.00%	-	0.73 (0.59 - 0.9)

Abbreviations: LR+, Likelihood ratio for a positive test result; LR–, likelihood ratio for a negative test result.

* The number of dichotomized variables was the cumulated top-ranked predictors from Table 3, including Hb, age and miR-486.

Table 5. Relationship between cumulated risk score and selected variables. (n=48)

Cumulated Risk Score	Total	Hb ≥ 10.7		Age ≥ 63		miR-486 ≥ 32.68	
		(n = 21)		(n = 28)		(n = 33)	
	n	n	%	n	%	n	%
S0	4	-		-		-	
S1	15	2	13.3	3	20.0	10	66.7
S2	20	10	50.0	16	80.0	14	70.0
S3	9	9	100.0	9	100.0	9	100.0

Table 6. Logistic regression for mild cognitive impairment in patients receiving dialysis.

Variable	OR (95%CI)	*P*
Univariate		
Hb	2.29 (1.13 - 4.64)	0.022
Age	1.06 (0.99 - 1.13)	0.106
miR-486	4.24 (1.14 - 15.79)	0.031
Multivariate		
Hb	2.74 (1.13 - 6.67)	0.026
Age	1.04 (0.96 - 1.13)	0.351
miR-4861	7.54 (1.47 - 38.6)	0.015
2-order interaction		
Hb*Age	1.01 (1.001 - 1.01)	0.019
Hb*miR-486	1.17 (1.03 - 1.32)	0.016
Age*miR-486	1.03 (1.004 - 1.05)	0.019
3-order interaction		
Hb*Age*miR-486	Omitted	-

Figures

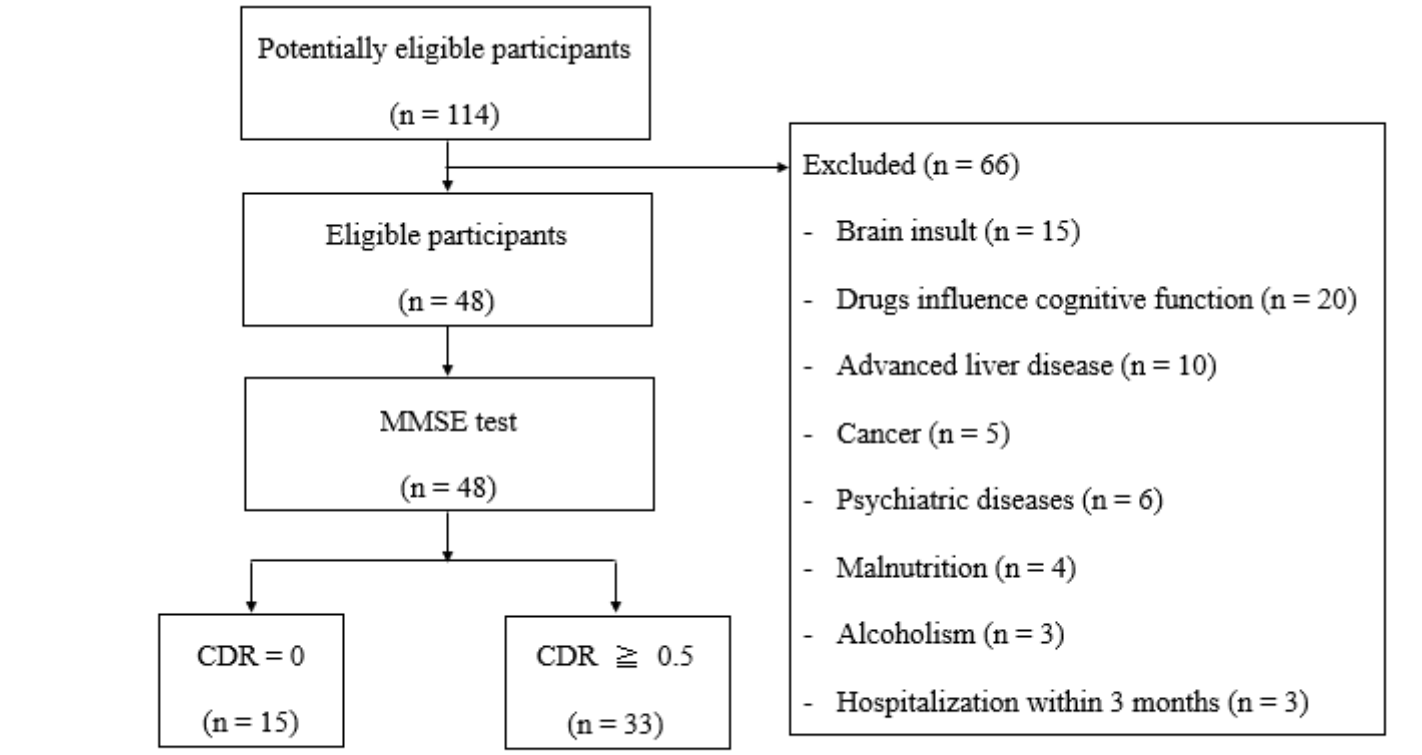

Figure 1

Participants flow diagram

Supplementary Files

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