Clinical assessment of nutritional status using the modified quantified subjective global assessment and anthropometric and biochemical parameters in patients undergoing hemodialysis in Macao

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Abstract

Objective: Malnutrition is widespread among patients undergoing hemodialysis and is linked to high morbidity and mortality rates. We evaluated the nutritional status and malnutrition markers in patients undergoing hemodialysis in Macao.

Methods: We performed a cross-sectional analysis of 360 patients in a hemodialysis center. The modified quantitative subjective global assessment (MQSGA), anthropometric indices and related biochemical test data were used to evaluate nutritional status.

Results: The sample's mean age was 63.47 ± 13.95 years. There were 210 well-nourished (58.3%), 139 mild-to-moderately malnourished (38.6%) and 11 severely malnourished (3.1%) patients. Older patients had a higher incidence of severe malnutrition, but there were no significant differences between diabetic and non-diabetic patients. Mid-arm circumference (MAC); mid-arm muscle circumference; body mass index; triceps skin fold thickness; serum albumin, creatinine and urea; and hemoglobin were all valid for assessing nutritional status. MAC and the serum albumin and creatinine concentrations significantly negatively correlated with MQSGA.

Conclusions: Malnutrition is commonplace in patients undergoing hemodialysis in Macao, but their nutritional status is not affected by diabetes. Serum creatinine, serum albumin and MAC, and especially pre-dialysis creatinine concentration, represent effective, readily available, and easily remembered screening measures of nutritional status for patients undergoing maintenance dialysis.

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Introduction

Patients undergoing hemodialysis often lack protein and energy reserves, which results in malnutrition.\(^1\)\(^-\)\(^4\) The evaluation of nutritional status is part of the routine care of patients undergoing maintenance hemodialysis. In patients with end-stage renal disease, malnutrition is one of the best predictors of morbidity and mortality. Malnutrition increases the risks of anemia, weakness, inadequate rehabilitation, infection and hospitalization, and ultimately leads to a higher incidence of mortality.\(^3\)\(^-\)\(^8\)

The primary etiology of malnutrition is multifactorial, and includes gastrointestinal dysfunction, insufficient intake of protein and energy, the accumulation of metabolic waste, psychological and economic factors, metabolic acidosis, protein loss, inadequate dialysis, secondary hyperparathyroidism, inflammation and infection, polypharmacy, insufficient activity, and impaired mastication and deglutition.\(^9\)\(^-\)\(^11\)

It is difficult to determine a patient’s nutritional status using a single method in clinical practice. There are several standard methods for the assessment of nutritional status, and body mass, body mass index (BMI) and biochemical indices, such as serum albumin concentration, have conventionally been used to determine nutritional status. Detsky \textit{et al.}\(^12\) were the first to establish a subjective global assessment (SGA) for the evaluation of nutritional status, and Kalantar-Zadeh \textit{et al.}\(^13\) subsequently developed the well-established modified quantitative subjective global assessment (MQSGA), with the motivation that a fully quantitative scoring system and a revamped malnutrition scoring system that uses the components of the conventional SGA would show superior performance.\(^13\) The MQSGA incorporates the benefits of the SGA, while also improving its reliability and precision, and providing a reasonably well validated method of determining nutritional status.

There have been few epidemiological studies of nutritional status in patients undergoing hemodialysis in Macau. Therefore, in this study, we aimed to assess the nutritional status of patients undergoing hemodialysis using standard assessment procedures, such as anthropometric measurements and biochemical parameters, to determine their relationships with MQSGA, and finally to evaluate potential markers of nutritional status.

Methods

We performed a descriptive-analytic cross-sectional study at Kiang Wu hospital, Macau. Before being enrolled in the study, patients who had been undergoing hemodialysis for at least 6 months agreed to complete all the nutritional assessments. The exclusion criteria were significant cardiovascular conditions, serious infections, cancer, and severe gastrointestinal or hepatic disease. The nutritional assessments were made in patients undergoing hemodialysis, who gave their written informed consent and whose medical records were analyzed.
anonymously. The study was conducted according to the principles of the Declaration of Helsinki and was approved by the Institutional Review Board of the Ethics Commission of Kiang Wu Hospital (approval number 2015-003).

Anthropometric measurements

Several anthropometric measurements were performed at the end of a hemodialysis session. The mid-arm circumference (MAC) and mid-arm muscle circumference (MAMC) were used to estimate muscle mass. Triceps skinfold thickness (TSF) was calculated using a skin fold caliper, which provides an estimate of body fat. MAMC was determined using the formula:

\[
\text{MAMC (cm)} = \frac{\text{MAC (cm)}}{C_{0.31415}} - \frac{\text{TSF (mm)}}{C_{2}}
\]

These measurements were made using a measuring tape. After each hemodialysis session, the dry mass of the participant was measured at least three times, and all the anthropometric measurements were made three times. BMI was calculated as end-hemodialysis body mass (kg) divided by the square of height (m²).

Evaluation of nutritional status

The nutritional status of the participants was evaluated using MQSGA. Body mass change, dietary intake, gastrointestinal symptoms, functional ability, comorbidities, subcutaneous fat mass, and signs of muscle wasting constitute the seven components of the MQSGA. Each parameter was scored between 1 (normal) and 5 (very severe), resulting in a total score ranging between 7 (normal) and 35 (severely malnourished). After physical testing, the participants were classified as having normal nutritional status (score 7 to 10), mild-to-moderate malnutrition (score 11 to 20) or severe malnutrition (score 21 to 35). The nutritional status of each participant was assessed independently by two physicians.

Laboratory testing

Urea clearance (Kt/V), serum albumin, hemoglobin, serum creatinine and urea nitrogen were measured before dialysis. A Cobas 6000 system (Roche Diagnostics, Basel, Switzerland) was used to measure the serum creatinine concentration, using an enzymatic method.

Statistical analysis

SPSS 22.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Continuous data are presented as mean ± standard deviation and were analyzed using Student’s t-test or ANOVA, followed by Tukey’s post-hoc test. Categorical data are displayed as number (percentage) and were analyzed using the chi-square test. The strengths of the relationships between MQSGA score and anthropometric or biochemical parameters were determined using Spearman’s correlation. The utility of independent parameters for the assessment of nutritional status was quantified using receiver operating characteristic curve (ROC) analysis, with MQSGA as the reference criterion. \( P \leq 0.05 \) was considered to represent statistical significance.

Results

Demographic data

We recruited 360 patients who were undergoing hemodialysis (204 men, 56.7%). Their mean age was 63.47 ± 13.95 years (range 22 to 94 years) and their median (interquartile range) length of hemodialysis 4.5 (2.9) years. Each dialysis session lasted a mean of 4.0 ± 0.2 hours. The mean dry body mass of the participants after hemodialysis was 56.42 ± 13.07 kg. Large proportions of the participants had been
diagnosed with diabetes (59.2%) or hypertension (23.9%).

Comparison of anthropometric and laboratory data between men and women

The anthropometric and laboratory data are shown in Table 1. The serum creatinine concentration of the male participants was higher than that of the female participants. The men also had higher values of all the anthropometric parameters and indices of nutritional status than the women, despite there being no other substantial differences in the other laboratory data between the men and women. (Table 1).

Comparison of diabetic and non-diabetic participants

A large proportion of the participants had been diagnosed with diabetes (59.2%). Therefore, we next aimed to determine whether there was a difference in the nutritional status of participants with and without diabetes. We found that there were no significant differences in the nutritional indices between these groups (see Table 2).

Anthropometric and laboratory data and nutritional status

The participants were categorized as having a normal nutritional status,

| Parameter       | Men (n = 204) | Women (n = 156) | P value |
|-----------------|--------------|----------------|---------|
| Height (m)      | 1.65 ± 0.63  | 1.53 ± 0.60    | 0.327   |
| Body mass (kg)  | 60.40 ± 12.76| 51.19 ± 11.57  | 0.459   |
| BMI (kg/m²)     | 22.00 ± 3.99 | 21.85 ± 4.38   | 0.575   |
| TSF (mm)        | 24.92 ± 9.54 | 23.35 ± 8.06   | 0.502   |
| MAC (cm)        | 26.92 ± 3.73 | 26.02 ± 4.09   | 0.397   |
| MAMC (cm)       | 19.09 ± 2.28 | 18.69 ± 2.44   | 0.502   |
| Albumin (µmol/L)| 539.6 ± 51.8 | 524.1 ± 51.6   | 0.949   |
| Creatinine (µmol/L) | 962.39 ± 201.89 | 774.3 ± 158 | 0.001   |
| Urea (µmol/L)   | 23.54 ± 5.42 | 23.17 ± 5.81   | 0.667   |
| Hemoglobin (g/L)| 105.48 ± 11.76| 103.73 ± 10.48 | 0.417   |
| MQSGA           | 10.88 ± 2.90 | 11.16 ± 2.84   | 0.915   |

Data are mean ± standard deviation.
Comparisons were made using Student's t-test.
MQSGA, modified quantitative subjective global assessment; MAC, mid-arm circumference; MAMC, mid-arm muscle circumference; TSF, triceps skinfold thickness, BMI, body mass index.

| Nutritional status | Participants with diabetes | Participants without diabetes | Total | χ² | P value |
|--------------------|---------------------------|-------------------------------|-------|----|--------|
| Normal             | 128 (35.6%)               | 82 (22.8%)                   | 210   |    |        |
| Mild-to-moderate malnutrition | 78 (21.7%)               | 61 (16.9%)                   | 139   |    |        |
| Severe malnutrition | 7 (1.9%)                  | 4 (1.1%)                     | 11    |    |        |
| Total              | 213 (59.2%)               | 147 (40.8%)                  | 360   | 0.90| 0.65   |

Data are number (percentage).
The chi-square test was used.
mild-to-moderate malnutrition, or severe malnutrition, using the MQSGA score. We found that 58.3% of the participants had a normal nutritional status (score 7 to 10), 38.6% had mild-to-moderate malnutrition (score 11 to 20) and 3.1% had severe malnutrition (score 21 to 35) (Table 3). The serum concentrations of albumin and creatinine decreased with the worsening of nutritional status ($P \leq 0.05$). Then, using a hierarchical analysis of nutritional status, we aimed to corroborate the anthropometric and biochemical findings and to identify parameters that could be used to evaluate nutritional status. BMI, MAC, MAMC, TSF, body mass, serum albumin, serum creatinine and even age were found to be associated with nutritional status ($P \leq 0.05$). This suggested that the lower the BMI, MAC, MAMC, TSF, body mass, albumin and creatinine of patients, and the higher the MQSGA score is, the more severe their malnutrition is. (see Table 3)

### Comparison of nutritional status among age groups of participants

We found that age correlated with nutritional status. Therefore, we next placed the 360 participants into four groups according to their age, and found that older patients had a higher incidence of severe malnutrition (see Table 4).

### Relationships of anthropometric and laboratory data with MQSGA

We next investigated the relationships of anthropometric and laboratory data with MQSGA (Table 5). The mean MAC, which assesses the thickness of subcutaneous fat and muscle, was $26.53 \pm 3.91$ cm, and there was a negative correlation between this and MQSGA ($r = -0.224; P = 0.001$). MAMC, which is an index of protein status, also negatively correlated with MQSGA ($r = -0.111; P = 0.035$). In addition, serum creatinine ($r = -0.203$, $P < 0.05$ vs. the normal nutrition group.)

**Table 3.** Demographic, anthropometric and laboratory data, and nutritional status of the participants after categorization according to MQSGA score.

| Parameter               | Total          | Normal nutrition | Mild-to-moderate malnutrition | Severe malnutrition | $P$ value |
|-------------------------|----------------|------------------|------------------------------|---------------------|-----------|
| Sample size             | 360            | 210 (58.3%)      | 139 (38.6%)                 | 11 (3.1%)           |           |
| Age, years              | 63.47 ± 13.95  | 61.01 ± 13.02    | 66.12 ± 10.11*              | 73.01 ± 11.03*      | 0.02      |
| Body mass (kg)          | 56.42 ± 13.07  | 58.05 ± 12.9     | 54.85 ± 13.03*              | 44.98 ± 7.59*       | 0.03      |
| Height (m)              | 1.60 ± 0.09    | 1.61 ± 0.08      | 1.59 ± 0.09                 | 1.57 ± 0.07         | 0.55      |
| BMI (kg/m²)             | 21.94 ± 8.95   | 22.5 ± 4.37      | 27.66 ± 4.01                | 18.28 ± 2.59*       | 0.01      |
| TSF (mm)                | 24.24 ± 8.95   | 25.50 ± 8.90     | 23.0 ± 8.66*                | 14.99 ± 4.57*       | 0.002     |
| Hemoglobin (g/L)        | 104.72 ± 11.25 | 106.5 ± 11.12    | 102.3 ± 10.9                | 102.3 ± 11.2        | 0.45      |
| MAC (cm)                | 26.53 ± 3.91   | 27.15 ± 3.77     | 25.93 ± 3.90*               | 22.18 ± 2.50*       | 0.003     |
| MAMC (cm)               | 18.92 ± 2.36   | 19.81 ± 2.67     | 17.56 ± 2.56*               | 14.58 ± 2.27*       | 0.001     |
| KT/V                    | 1.61 ± 0.31    | 1.62 ± 0.3       | 1.63 ± 0.32                 | 1.48 ± 0.39         | 0.34      |
| Urea (µmol/l)           | 23.38 ± 5.59   | 23.98 ± 5.1      | 22.76 ± 5.8                 | 21.48 ± 9.7         | 0.23      |
| Creatinine (µmol/L)     | 860.48 ± 198.90| 887.8 ± 197.7    | 832.2 ± 192*                | 696.3 ± 195.8*      | 0.01      |
| Albumin (µmol/L)        | 533.0 ± 52.2   | 543.6 ± 49.4     | 521.8 ± 56.3*               | 466.4 ± 74.9*       | 0.002     |
| MQSGA                   | 11.01 ± 2.87   | 9.34 ± 0.90      | 12.64 ± 1.91                | 21.91 ± 1.81        |           |

Data are mean ± standard deviation and were compared using ANOVA, followed by Tukey’s post-hoc test.

* $P < 0.05$ vs. the normal nutrition group.

MQSGA, modified quantitative subjective global assessment; MAC, mid-arm circumference; MAMC, mid-arm muscle circumference; TSF, triceps skinfold thickness; BMI, body mass index.
P = 0.002), serum albumin (r = -0.258, P = 0.001), BMI (r = -0.156, P = 0.003) and TSF (r = -0.201, P = 0.001) all negatively correlated with MQSGA (Table 5).

### Identification of markers of nutritional status

We have shown above that serum creatinine, serum albumin and MAC were relatively good biomarkers of nutritional status. Therefore, we next used ROC curve analysis to determine whether these parameters would represent clinically useful predictors of nutritional status, using MQSGA as the reference standard (Figure 1). The areas under the curves (AUCs) were 0.649 (95% confidence interval [CI] = 0.531–0.798) for creatinine, 0.597 (95% CI = 0.538–0.656) for MAC, and 0.562 (95% CI = 0.50–0.624) for albumin. High values of creatinine concentration, albumin concentration and MAC were shown to be appropriate predictors of good nutritional status (P < 0.05). The use of a threshold creatinine concentration of 699 µmol/L provided 91.9% sensitivity and 75.1% specificity for the prediction of malnutrition; the use of a threshold albumin concentration of 369 µmol/L provided 80.5% sensitivity and 66.7% specificity for the prediction of malnutrition; and the use of a threshold MAC value of 24.0 cm provided 83.6% sensitivity and 73.6% specificity for the prediction of malnutrition.

### Discussion

Malnutrition is a significant concern for patients undergoing hemodialysis all over the world. Protein-energy wasting is a leading cause of mortality in hemodialysis patients,\(^1\) with an incidence of 18.0% to 75.0%.\(^2\) In the present study, we aimed to use the MQSGA to determine the nutritional status of patients undergoing hemodialysis in a center in Macau. We found that the overall malnutrition rate was 41.7%, with mild-to-moderate malnutrition accounting for 38.6% and severe malnutrition accounting for 3.1%. In Taiwan, the prevalence of malnutrition in patients undergoing hemodialysis is 45%.\(^3\) However, the nutritional status of Taiwanese people is far superior to that of

| Table 4. Comparison of the nutritional status of participants in various age groups. |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|---------------|-----------------|-----------------|
| Nutritional status                          | ≤44 years       | 45–59 years     | 60–74 years     | ≥75 years       | Total         | χ²value   |
| Normal                                      | 23 (74.2%)      | 65 (60.2%)      | 86 (65.1%)      | 36 (40.4%)      | 210           |            |
| Malnutrition                                | 8 (25.8%)       | 43 (39.8%)      | 46 (34.9%)      | 53 (59.6%)      | 150           |            |
| Total                                       | 31              | 108             | 132             | 89              | 360           | 17.59      |
| The χ² test was used.                       |                 |                 |                 |                 |               |            |

| Table 5. Relationships of anthropometric and laboratory data with MQSGA in patients undergoing hemodialysis. |
|----------------------------------------------------------|-------------|-------------|
| Parameter                        | MQSGA       |             |
|---------------------------------|-------------|-------------|
| BMI (g/m²)                      | -0.156      | 0.003       |
| TSF (mm)                        | -0.201      | 0.001       |
| MAC (cm)                        | -0.224      | 0.001       |
| MAMC (cm)                       | -0.111      | 0.035       |
| Albumin (µmol/L)                | -0.258      | 0.001       |
| Creatinine (µmol/L)             | -0.203      | 0.002       |
| Hemoglobin (g/L)                | -0.140      | 0.008       |
| Urea (µmol/L)                   | -0.131      | 0.013       |
| Normalized creatinine           | -0.091      | 0.124       |

N = 360.
The r-values are Spearman’s correlation coefficients.
BMI, body mass index; MAC, mid-arm circumference; MAMC, mid-arm muscle circumference; TSF, triceps skinfold thickness.

P = 0.002), serum albumin (r = -0.258, P = 0.001), BMI (r = -0.156, P = 0.003) and TSF (r = -0.201, P = 0.001) all negatively correlated with MQSGA (Table 5).
people in mainland China: in one multicenter study conducted in southern China, 68% of the patients had malnutrition and 8% had severe malnutrition. The annual mortality rate of Chinese patients undergoing hemodialysis has been estimated to be ~10%.19

In routine clinical practice, the assessment of nutritional status is often ignored, and certain assessment metrics are not particularly accurate. Serum albumin, for example, is a valuable index of visceral protein storage that can be used to determine whether a patient is at risk of malnutrition. However, this is influenced by non-nutritional factors, such as edema, liver disease and chronic inflammation, and when malnutrition develops, it appears to worsen over a long period of time. Owing to edema and variations in tissue water content, some widely used anthropometric indices, such as BMI, are also not reflective of nutritional status.

The MQSGA is a helpful tool for the assessment of the nutritional status of a patient undergoing hemodialysis. MQSGA is closely associated with anthropometric parameters, according to Kalantar-Zadeh et al.14 Consistent with this, in the present study, MAC, BMI, serum albumin, serum creatinine, urea, MAMC, TSF and BMI were all found to be associated with nutritional status (Tables 3 and 5). The lower the value of each of these, the worse was the nutritional status of the participants ($P < 0.05$), and therefore, each may

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**Figure 1.** Receiver operating characteristic (ROC) curve analysis of the assessments of nutritional status, using modified quantitative subjective global assessment (MQSGA) as the reference standard (*$P < 0.05$).** For each parameter, sensitivity is plotted against 100 specificity. An ideal test would have 100% sensitivity and 100% specificity and reach the upper left corner of the graph, whereas a test with no diagnostic value would lie along the diagonal between the lower left corner and the upper right corner.
represent a predictor of nutritional status. Although BMI is not a sensitive indicator of malnutrition, it represents a simple and valuable tool for the assessment of nutritional status.\textsuperscript{20} TSF is indicative of body fat mass, MAC may represent the level of muscle protein storage and MAMC represents the thickness of the subcutaneous fat and muscle. Decreases in serum albumin and creatinine represent reductions in visceral and muscle protein storage, and imply protein deficiency. Therefore, these parameters may be suitable for use as part of a clinical screening index for the assessment of the nutritional status of patients undergoing hemodialysis. In particular, we showed that creatinine, albumin and MAC all had relatively strong relationships with nutritional status and were more predictive of nutritional status than the other parameters assessed in the present study.

Although albumin is not a sensitive marker of malnutrition, it is still widely used in clinical settings to assess nutritional status.\textsuperscript{21} Therefore, more accurate and convenient means of assessment are sought. Serum albumin concentration had a relatively high sensitivity (80.5\%) and specificity (66.7\%) for the prediction of malnutrition in the present study, using a threshold of 369 m\(\text{mol/L}\). The findings with respect to MAC were consistent with MQSGA, and ROC analysis revealed that MAC is an excellent diagnostic tool for malnutrition, using a threshold value of 24.0 cm, providing 83.6\% sensitivity and 73.6\% specificity. However, creatinine was a stronger predictor of malnutrition than either MAC or albumin, with a threshold value of 699 mol/L being associated with 91.9\% sensitivity and 75.1\% specificity. There are various existing approaches for the assessment of the nutritional status of patients undergoing hemodialysis, and each has its own drawbacks.\textsuperscript{22} We and others\textsuperscript{23,24} have shown that serum creatinine is a strong predictor of nutritional status. Changes in the pre-dialysis serum creatinine concentration of individual patients with time have been linked to mortality by Kalantar-Zadeh et al.\textsuperscript{25} When kidney function is poor or absent in patients undergoing hemodialysis, creatinine concentration may represent an appropriate surrogate for muscle mass. Normalization of the creatinine concentration to body surface area allows comparisons to be made between individuals with differing protein intake and metabolism.\textsuperscript{26} Therefore, we hypothesize that muscle mass, as well as clearance during dialysis, affects the pre-dialysis creatinine concentration. This would mean that treatments aimed at increasing muscle mass, such as diet or exercise, could be monitored using pre-dialysis creatinine concentration.

The present findings also show that the prevalence of malnutrition in elderly patients is substantially higher than that in younger patients, possibly because of the poorer economic and social status of the older patients (see Table 4). The majority of these resided in nursing homes and had no family to look after their diet. Furthermore, many were affected by chronic diseases. According to previous studies, the prevalence of malnutrition among elderly patients undergoing hemodialysis is higher than that among younger patients.\textsuperscript{27} The male participants tended to have higher values of all the anthropometric parameters and nutritional status indicators than women in the present study, but there were no significant differences, except with respect to serum creatinine (see Table 1). Thus, the nutritional status of patients undergoing hemodialysis was unaffected by their sex, as previously shown,\textsuperscript{28} or the length of time they had been undergoing dialysis. We also found that there was no substantial difference in Kt/V between well-nourished and malnourished participants. It has previously been shown that for
every 0.1 increase in Kt/V, the mortality rate drops by 2%, and it has been hypothesized that increasing the frequency of hemodialysis would help improve the efficiency of the dialysis regimen, thereby improving the nutritional status and reducing the mortality rate of the patients.\textsuperscript{29}

It is also worth noting that we did not identify a relationship between diabetes and malnutrition in the present study, which should be further investigated. In contrast, a previous study showed that patients with diabetes who were undergoing hemodialysis had a higher incidence of malnutrition and a lower survival rate than those without diabetes. However, the higher risk of death that was associated with diabetes was not linked to malnutrition.\textsuperscript{30}

Anthropometric measurements are inexpensive and do not require blood sampling, such that they can be used as the first line of nutritional assessment for patients undergoing dialysis in a routine clinical setting. MQSGA is similar to these, in that it is non-invasive and inexpensive. However, there is a significant issue with its use: that of “dry mass” and excessive fluid accumulation in the body, which affects anthropometric parameters in patients undergoing hemodialysis. Therefore, a combination of creatinine, albumin and MAC, or another combination, may be more useful for the comprehensive assessment of nutritional status or as a clinical screening index for the nutritional status of patients undergoing hemodialysis, and might facilitate the earlier detection of malnutrition and the institution of appropriate therapies.

In conclusion, malnutrition is prevalent in patients undergoing hemodialysis in Macau. Serum creatinine, serum albumin, BMI, MAC, MAMC and TSF may be appropriate and practical means of assessing nutritional status. In particular, creatinine, albumin and MAC, which are surrogates of MQSGA, may represent predictors of nutritional status, despite not being strongly correlated with MQSGA. Thus, any single parameter does not represent a reliable marker of nutritional status in patients undergoing dialysis. In contrast, the use of multiple indices for the assessment of nutritional status should help clinicians diagnose malnutrition in patients undergoing dialysis earlier. More importantly, we found that routine pre-dialysis creatinine concentration measurements every month represent inexpensive, readily available, and easily remembered screening measures of nutritional status for patients undergoing maintenance dialysis.

**Declaration of conflicting interest**
The authors declare that there is no conflict of interest.

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