Differences between measured total nitrogen losses in spent peritoneal dialysate effluent and estimated nitrogen losses

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Abstract

Objective

Kidney dialysis patients treated by peritoneal dialysis (PD) are at increased risk of muscle wasting and clinical guidelines recommend assessing dietary intake, by calculating protein equivalent of nitrogen appearance (PNA) to assure protein sufficiency. The PNA equations were developed many years ago, and we wished to re-evaluate them by comparing estimated and measured peritoneal nitrogen losses.

Design

Cross sectional observational cohort study

Setting

Outpatient peritoneal dialysis centre of a University Hospital

Subjects

67 peritoneal dialysis patients, 61.2% male, median age 67.3 (53.2-79.4) years

Intervention

Measurements of the nitrogen content of 24 hour spent peritoneal dialysate, by automated chemiluminescence analyser compared to estimates of nitrogen losses based on dialysate urea loss using the Bergström, Randerson and Blumenkrantz equations.
Results

Measured total dialysate nitrogen was more than urea nitrogen equivalent, 5.79±4.07 vs 2.66±1.67 g/day (p<0.001). Each equation has an inflation factor to compensate for non-urea protein losses, however measured nitrogen loss was 27.7 (15.5-59.6) vs Bergström 16.5 (9.8-27.1), Randerson 16.4 (9.8-27.3) and Blumenkrantz 12.9 (7.9-25.4) g/day, p<0.001. Bland Altman analysis demonstrated systematic bias with increasing under-estimation by these equations with increasing measured nitrogen losses (r=0.74, p<0.001).

Conclusion

Our findings demonstrate that at higher protein losses, the currently used predictive equations underestimate the amount lost. It is important to attempt to compensate iatrogenic protein loss by recommending the appropriate intake of dietary protein to patients, in an attempt to minimise muscle wasting. This discrepancy may have arisen because of the characteristics of newer PD prescriptions and change in patient demographics. We propose a new equation PNA g/day = 0.31 x (urea loss mmol) + 7.17, which will require prospective validation in additional studies.

Introduction

Patients with chronic kidney disease (CKD) are at increased risk of losing muscle mass. The reasons for this are many [1] and include episodes of metabolic acidosis during progression of CKD which switch on the proteasome
pathway of muscle protein breakdown, low protein diets which aim to slow CKD progression [2], bone and mineral disorders and background inflammation which, itself, promotes muscle loss [3]. In view of this, clinical guidelines recommend the regular assessment of dietary protein intake, with nutritional targets designed to reduce muscle breakdown and loss [4,5]. Assuming that a patient with CKD is not losing muscle mass if they are in neutral protein balance, then a useful laboratory measure of this is nitrogen balance, which is the difference between protein (i.e. nitrogen) intake and nitrogen losses. The protein equivalent of nitrogen appearance (PNA) has been traditionally estimated from combined urinary and peritoneal urea losses and it is assumed that urea represents a constant proportion of total nitrogen excreted [6]. In reality this is not always the case because nitrogen excretion switches between urea and ammonia, depending on fluctuating acid-base balance [7].

Thus, a relative increase in the proportion of nitrogen excreted as ammonia may be undetected, and urea excretion will yield an underestimate of nitrogen excretion. This would represent a false positive nitrogen balance which may mask concomitant muscle protein loss.

Despite this, three equations are currently used in clinical practice, all derived from small studies in peritoneal dialysis (PD) patients treated by continuous ambulatory peritoneal dialysis (CAPD) [8-10], with the largest study based on 23 measurements from 12 patients [8]. In those studies, the equations were derived from correlation of urea excretion and of total nitrogen excretion measured by the Kjeldahl method. This technique may not account for all
forms of nitrogen resistant to acid conversion to ammonia. In contrast, automated chemiluminescence analysis is superior because all forms of nitrogen are converted to nitric oxide, during pyrolysis at 1100°C [11]. An added advantage is that liquid samples require only simple dilution and can be analysed in duplicate within 4 minutes.

As patient demographics and PD prescriptions have changed over time we wished to determine whether there were differences in determining PNA using these equations, by measuring the excretion of nitrogen in urine and peritoneal dialysate effluent in the form of either urea or total nitrogen. In effect we have repeated the original studies [6,8,9] but with patients undergoing modern renal therapies.

Methods

We measured urea in twenty-four hour collections of spent dialysate effluent [1,2], using a standard laboratory analyser (P module analyzer, Roche Integra, Roche diagnostics, Lewes, UK [12]), and total nitrogen by chemiluminescence. In brief samples are pyrolysed at 1100°C in a mixture of oxygen and argon to quantitatively convert all nitrogen compounds to nitric oxide, and then reacted with ozone. Reaction with ozone yields nitrogen dioxide in which an electron relaxes to ground state emitting a photon. This chemiluminescence is measured by a photomultiplier tube [11,13]. Peritoneal dialysate protein was measured using pyrogallol red-molybdate (PRM) (Hitachi 726 auto analyser, Maidenhead UK). This method is linear up to 2.14 g/L, and
higher concentration samples were diluted to bring them into range [12]. C-
reactive protein (CRP) was measured using the same assay as the UK National
Amlyoid centre, with values reported down to < 1 mg/L, and haemoglobin was
measured by an automated counter (Sysmex XN900, Sysmex Corporation, Kobe,
Japan) [14]. Patients used standard lactate containing glucose dialysates (Baxter
Health Care, Deerfield, Illinois, USA). In addition to 13.6 g/dL dextrose, 31
(59.6%) used 22.7 g/dL dextrose, median 4.0 (0-7.75) L/day, and 42 (80.8%)
used 1.5 (0.6-2.0) L/day 7.5% icodextrin. Body composition was measured by
multifrequency bioelectrical impedance (MFBIA) (InBody 720, Seoul, South
Korea) [15]. Patients had not had peritonitis or other infections, or hospital
admissions within the previous 8 weeks. MFBIA [16] was measured in a
previously reported standardised manner; first patients were asked to empty
the bladder and then peritoneal dialysate was drained out, as ascites and
peritoneal dialysate can potentially alter bioimpedance derived body composition
measurements [17]. Patients with amputations, pregnancy, and those with limb
paralysis were excluded. Patient demographics and ethnicity were obtained
from the Hospital patient records.

PNA was calculated using the following equations [4,5]:

Bergström [6]

20.1 + 7.50 Urea Nitrogen Appearance g/24 hours

Randerson [8],

15.7 + 7.47 Urea Nitrogen Appearance g/24 hours

and Blumenkrantz [9]
34.6 + 5.86 Urea Nitrogen Appearance g/24 hours

Dialysate urea nitrogen was determined by adjusting for the nitrogen content of urea: 1 mole urea being equivalent to 28g nitrogen.

This project was registered with the UK Integrated Research Application System (IRAS) reference number 191812/893749/14/564 was approved by the National Research Ethics (Manchester) and the Hospital Research and Development Service and complied with NHS guidelines (UK NHS guidelines for clinical audit and service development). Individual consent was waived as only waste samples were analysed. In keeping with the Hospital Trust policy no patient identifiable data was used.

**Statistical analysis**

Data is presented as mean ± standard deviation, median (interquartile range), or percentage. Standard statistical tests: Wilcoxon rank sum pair test Anova, or Kruskal Wallis) with appropriate post hoc corrections made for multiple testing (Tukey or Dunn), where appropriate. Bland Altman analysis was used to determine agreement between methods. Statistical analysis used Prism 7.0 (Graph Pad, San Diego, USA) and SPSS 24 (IBM, Armonk, New York, USA).

Statistical significance was taken as p<0.05.

**Results**
We measured urea and total nitrogen in 67 PD outpatients attending for the assessment of peritoneal membrane function, and dialysis adequacy (Table 1). The median number of peritoneal dialysate exchanges prescribed was 5.5 (4.0-6.0)/day with a median peritoneal dwell volume of 2.0 (2.0-2.0) L. Total nitrogen (measured by chemiluminescence) was greater than that for the urea nitrogen equivalent, 5.79±4.07 vs 2.66±1.67 g/day although there was a strong linear relationship between the two (Figure 1). The difference represents the non-urea nitrogen losses (e.g. ammonia, protein), which are approximately 55% of total nitrogen loss.

Each of the three equations converts urea nitrogen in spent dialysate effluent to total nitrogen on the basis of the regression equation derived from previous studies [6,8,9]. In each equation, the intercept and slope of the relationship between urea nitrogen and total nitrogen (which includes other nitrogen losses in the dialysate - an "inflation factor") differed. We then compared total nitrogen lost in the spent dialysates and the urea nitrogen equivalent adjusted by the individual inflation factor. For all three equations there was a positive correlation with total nitrogen losses, r=0.74, p<0.001. Bland-Altman analysis revealed a systematic bias for estimated total nitrogen when compared to measured total peritoneal nitrogen losses. At lower urea losses, total nitrogen loss was overestimated, whilst at higher peritoneal urea losses, total nitrogen loss was underestimated (Figures 2-4). The difference between measured total nitrogen and that estimated by each of the three equations differed significantly (Bergström -12.5 (-30.8 -4.1), Randerson -12.5 (-
30.8 -), Blumenkrantz - 16.2 (-36.6 -6.5), p<0.001). There was no correlation between this difference in peritoneal nitrogen loss and either serum urea (r=0.11, p=0.41), or total protein (r=0.06, p=0.66), but the difference between measured nitrogen losses and estimated losses increased with increasing total peritoneal dialysate protein loss (r=0.37, p=0.004), total drained dialysate volume (r=0.34, p=0.025), and greater muscle mass (r=0.28, p=0.026), but not body weight (r=0.13,p=0.29) or body fat mass (r=0.14, p=0.28).

Using our own data then the regression equation between PNA and daily peritoneal urea losses was PNA g/day = 0.31 (urea loss mmol) + 7.17. On Bland Altman analysis, this equation did not have the systematic bias of under estimating protein losses when patients had higher losses (Figure 5).

Discussion

Current clinical guideline recommend that PD patients should increase their dietary protein intake compared to those with chronic kidney disease to compensate for peritoneal protein losses [4,5]. These patients are potentially at increased risk of muscle wasting [1,2], and muscle wasting is an established risk factor for increased mortality [18]. Dietary protein intake is an important factor in maintaining muscle mass in the face of inflammation and other factors which stimulate net muscle protein breakdown. This is because amino acids released from skeletal muscle are either incorporated into acute phase proteins synthesised in the liver or oxidised for energy production with excretion of the amino group as urea or ammonia. In this situation, even though net muscle
protein breakdown is increased, the hope is that an adequate protein intake will provide substrate for muscle protein synthesis. Whereas the body has fat stores which can act as an energy store, the body holds no such equivalent store of protein which can provide amino acids and as such dietary protein intake is essential to prevent muscle loss.

Clinical guidelines recommend that PD patients should be assessed for dietary protein intake [4,5], and although dietary records and patient recall can be used, most dialysis centres use protein nitrogen accumulation (PNA) in clinical practice. PNA can be estimated by a number of equations [4-6], based on urea losses in spent peritoneal dialysate and urine, with an inflation factor to account for losses of proteins, small peptides and amino acids, and then a correction factor for other protein losses, including those from the gastrointestinal tract, and loss of skin, estimated to average around 1.3 g/day, although faecal losses may vary between 0.52-2.8g/day [6,8,10].

As discussed, the studies on which the equations for calculating PNA are based come from a limited number of small studies of patients treated by continuous ambulatory peritoneal dialysis (CAPD) [8,9,10]. Previous reports have shown that there are differences between the three most commonly used equations, and there is no universal consensus as to whether any one of these equations has superiority [6]. We found that all three equations under- estimated measured nitrogen losses with increasing losses, with the difference increasing from 4.4±8.5 to those with a PNA < 40 g/day to 33.9±13.3 g/day for those with a PNA > 40 g/day. This difference was greater with increasing volume
of drained peritoneal dialysate. We propose some explanations for this discrepancy. Whereas the equations used to estimate PNA were derived from studies using CAPD patients, who used between 6-8 litres of dialysate, the trend in Europe and North America now is to use automated peritoneal dialysis cyclers, using much higher volumes of dialysate, and in our study almost two thirds of patients were treated by APD cyclers. In addition, the difference between measured and estimated nitrogen losses was greater with increasing dialysate protein losses, and although these equations have an inflation factor to compensate for protein losses, this was based on lower dialysate volumes. Total nitrogen includes not only proteins and urea, but also small peptides, nucleotides, amino acids, organic acids, uric acid, nitrates and nitrites. However, even making allowance for some of these compounds (which will be increased in the present peritoneal dialysates) the Bergström, Randerson and Blumenkrantz equations under estimate nitrogen losses with increasing losses, and as such would under estimate nPNA. However, of more concern in patients with the lowest dialysate nitrogen losses, these equations over-estimated losses in a proportion of these patients. This group of patients would have the lowest PNA, and as such be at greater risk of muscle loss due to lower dietary protein intake. From the viewpoint of clinical risk, an overestimate of dietary intake in patients with the lowest PNA may prevent recognition of dietary protein inadequacy or delay a clinical decision to provide additional nutritional support.

We re-evaluated the standard equations used to estimate dietary protein intake due to the increasing use of automated peritoneal dialysis in our own
centre. Our study is based on a relatively small heterogeneous patient cohort. We need to prospectively evaluate our equation, as this may well have to be modified for age, gender, ethnicity, physical activity and dietary variations, for example vegans [19,20,21]. As previous reports have demonstrated that age, gender, and ethnicity all potentially have an effect on body composition, especially muscle mass. In addition, energy expenditure also has been shown to have an effect on body composition muscle mass [22,23].

The development of alternative methods to measure nitrogen losses in body fluids has allowed us to re-examine the estimation of PNA, using the three most commonly used equations, in peritoneal dialysis patients. These equations were based on a series of small studies in a generally younger cohort of patients treated by CAPD. Over the course of time there have been changes in patient demographics and PD prescriptions. By measuring total nitrogen, we have found that these equations developed more than 20 years ago may underestimate higher protein losses. We developed an equation from our data that does not have systematic bias, but this will need to be prospectively validated in separate patient cohorts, and it may be that different equations will be required for patients treated by continuous ambulatory peritoneal dialysis and those using cycler PD prescriptions.

Practical application

When estimating dietary protein intake by calculating the nitrogen appearance rate in peritoneal dialysis patients using the Bergström, Randerson or
Blumenkrantz equations, then these patients underestimate protein nitrogen losses in patients with greater protein nitrogen losses, and so underestimate dietary protein intake.

The authors have no conflict of interest. The data presented in this paper has not been previously published in part or full form.
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5. NKF-DOQI CLINICAL PRACTICE GUIDELINES FOR PERITONEAL DIALYSIS ADEQUACY. Assessment of Nutritional status. Am J Kid Dis 2007; 30(3 Suppl 2), S125-9


Figure 1. Total nitrogen measured in 24 hour spent dialysate compared to urea nitrogen equivalent.

Figure 2. Bland Altman plot daily mean peritoneal nitrogen loss, and difference between measured total nitrogen and estimated peritoneal urea nitrogen equivalent losses using the Bergström equation [10]. Dotted line - mean bias, dashed and dotted line 95% limits of association.

Figure 3. Bland Altman plot daily mean peritoneal nitrogen loss, and difference between measured total nitrogen and estimated peritoneal urea nitrogen equivalent losses using the Randerson equation [8]. Dotted line - mean bias, dashed and dotted line 95% limits of association.

Figure 4. Bland Altman plot daily mean peritoneal nitrogen loss, and difference between measured total nitrogen and estimated peritoneal urea nitrogen equivalent losses using the Blumenkrantz equation [9]. Dotted line - mean bias, dashed and dotted line 95% limits of association.

Figure 5. Bland Altman plot daily mean peritoneal nitrogen loss, and difference between measured total nitrogen and estimated peritoneal urea nitrogen equivalent losses using data obtained from this study. Dotted line - mean bias, dashed and dotted line 95% limits of association.
Figure 1

The graph shows the relationship between spent dialysate urea nitrogen (g/day) and spent dialysate total nitrogen (g/day). The correlation coefficient (r) is 0.73, and the p-value is less than 0.001.
Table 1. Patient demographics, dialysis prescriptions, body composition and laboratory investigations. Number (no), Continuous ambulatory peritoneal dialysis (CAPD), Duration of PD treatment (vintage) months, Results expressed as integers, mean ± standard deviation, median (interquartile range) or percentage.

<table>
<thead>
<tr>
<th>variable</th>
<th>Number (no), (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male - number, (%)</td>
<td>41 (61.2)</td>
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<tr>
<td>Age, years</td>
<td>67.3 (53.2-79.4)</td>
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<tr>
<td>Ethnicity no (%)</td>
<td></td>
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<tr>
<td>White</td>
<td>31 (46.3)</td>
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<tr>
<td>Black</td>
<td>16 (23.9)</td>
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<tr>
<td>Asian</td>
<td>20 (29.9)</td>
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<tr>
<td>Diabetes mellitus - no.(%)</td>
<td>26 (38.8)</td>
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<tr>
<td>Body mass index kg/m²</td>
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<tr>
<td>Skeletal muscle mass kg</td>
<td>26.7±6.7</td>
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<tr>
<td>% Body fat</td>
<td>32.6±10.2</td>
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<tr>
<td>Haemoglobin, g/dL</td>
<td>10.9±13.9</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>37.7 ±3.2</td>
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<tr>
<td>C reactive protein mg/L</td>
<td>4.0 (2.0-12.3)</td>
</tr>
<tr>
<td>Serum calcium mmol/L</td>
<td>2.28 ±0.17</td>
</tr>
<tr>
<td>Serum phosphate mmol/L</td>
<td>1.63 ±0.17</td>
</tr>
<tr>
<td>Dialysis vintage, months</td>
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<td>Peritoneal equilibrium test no (%)</td>
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<tr>
<td>Slow Average</td>
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<td>CAPD no (%)</td>
<td>23 (34.3)</td>
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Data Statement
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