Pseudohypernatremia and pseudohyponatremia: a linear correction

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ABSTRACT

Background. Serum sodium is commonly measured by direct potentiometry (D_{Na}), in blood gas panels, or indirect potentiometry (I_{Na}), in metabolic panels run on chemistry analyzers. Abnormal values of the serum non-water fraction interfere with I_{Na}, with low values causing pseudohypernatremia (I_{Na} > D_{Na}) and high values causing pseudohyponatremia (I_{Na} < D_{Na}). Previous attempts to derive a linear correction for the difference between I_{Na} and D_{Na} (ΔNa) arising from non-water bias—using serum total protein (TP) or albumin (ALB) to represent the non-water fraction—have yielded inconsistent results, possibly owing to differences in sample inclusion criteria, analytic platforms and statistical approach.

Methods. We quantified the effects of TP and ALB on ΔNa in 774 critical care patients with closely timed metabolic and gas panels, adjusting for other known effects.

Results. ΔNa varied inversely with TP, ALB, and the glucose difference between chemistry and gas panels (ΔGlu), and directly with pH and bicarbonate. The effect of TP on ΔNa was essentially linear, but that of ALB was not; hence, further analysis focused on TP. By multiple linear regression, ΔNa decreased by 0.64 ± 0.06 mEq/L for each 1 g/dL increase in TP, adjusted for regression to the mean; the TP effect was slightly steeper (0.69 ± 0.06 mEq/L), when adjusted for bicarbonate instead of pH.

Conclusions. For each 1 g/dL rise or fall in TP, clinicians may find it useful to adjust I_{Na} by 0.7 mEq/L in the same direction in order to correct I_{Na} for non-water bias.

Keywords: artifact, pseudohypernatremia, pseudohyponatremia, sodium, total protein

INTRODUCTION

Precision and accuracy in the measurement of serum sodium concentration are essential for the diagnosis and safe treatment of the dysnatremias [1]. In the era when serum sodium was measured by flame photometry (F_{Na}), clinicians were aware of pseudohyponatremia, a spuriously low F_{Na} resulting from the water exclusion effect, which occurs when the percent of a serum sample’s volume occupied by the substances which exclude water and electrolytes—i.e. lipids and colloidal proteins—exceeds the usual value of 7% [2–4]. The opposite artifact, pseudohypernatremia, occurring when the non-water fraction falls below normal [4–8], was overlooked by clinicians, even though a version of this artifact, resulting from sample deproteination by filtration, had been described long before the advent of flame photometry [9].

Currently sodium is measured with ion-selective electrodes, using either the indirect method (I_{Na}) on automated chemistry analyzers, or the direct method (D_{Na}) on blood gas analyzers [4, 6]. Since the indirect method involves pre-analytic dilution and assumes a non-water fraction of 7%, it retains the very same sensitivity to the water exclusion effect as the older flame method [4, 6, 10–12]. Consequently, an entirely spurious difference between simultaneous I_{Na} and D_{Na} measurements (ΔNa) may arise when the non-water fraction of serum is not 7% and, similarly, between sequential I_{Na} measurements if the non-water fraction changed between measurements. It would be useful indeed if this frequent source of bias could be corrected by a simple linear equation based on the serum concentration of either of the commonly measured colloid levels, albumin (ALB) and total protein (TP), in a manner similar to...
the adjustment of serum sodium for serum glucose [13], and to the adjustment of the anion gap and serum total calcium for ALB [14, 15].

A number of theoretical and empirical studies have attempted to quantify the effect of changes in either ALB or TP on either $I_{Na}$ or $F_{Na}$ or ANa [5, 6, 11, 12, 16–22] (Supplementary data, Table S1). The observational studies produced disparate estimates of these effects and even differ on whether the effects are linear or not. While this heterogeneity may stem from differences in study characteristics (study size, enrollment criteria, blood sample type, analytic platforms), it also may reflect the potentially inadequate handling of important statistical issues. For example, the studies are largely silent on the validation of modeling assumptions, and on their approaches to the spurious variation that results from regression to the mean [23], and, as noted by Jones and Twomey [20], to repeat (i.e. non-independent) measures [24] (Supplementary data, Table S1). The one study, by Dimeski et al. [21], that clearly addressed those statistical issues, treated TP only as a categorical variable, and did not examine ALB at all. Consequently, a statistically valid linear correction for non-water bias based on either TP or ALB remains to be established. Additionally, none of these studies adjusted for possible confounding by the tendency of increasing pH and/or bicarbonate concentrations to depress $D_{Na}$ and thus increase ANa [8, 25–28], even though disturbances of acid-base status are common in critically ill patients.

The purpose of the present study was to derive linear estimates of the effects TP and ALB on ANa in a large set of retrospectively collected pairs of $I_{Na}$ and $D_{Na}$ measurements, all made under 20 min apart in a critical care setting over a 3-year period, with testing for nonlinearity, avoidance of repeat measures, and adjustment for regression to the mean and for other known influences on ANa.

**MATERIALS AND METHODS**

**Patients**

All patients admitted to a critical care unit (intensive, coronary, surgical) of the Veterans Affairs New York (NY) Harbor Healthcare System (consisting of NY and Brooklyn (BK) campuses) between 1 January 2009 and 31 December 2011 were eligible. The protocol was approved by the institutional review board.

**Analytic methods**

Metabolic panel measurements of serum $I_{Na}$, glucose (sGlu), total carbon dioxide (tCO$_2$), TP and ALB were performed on the ADVIA Chemistry System 1650 (A1650) analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY) from 1 January 2009 until 31 March 2011 at the BK campus and until 27 May 2011 at the NY campus, after which the ADVIA Chemistry System 1800 (A1800) was used. The $I_{Na}$ assay is calibrated daily, reports results as whole numbers and has a linear range of 100–200 mEq/L; the expected coefficient of variation (CV) varies by analyzer (A1650: 1.8% A1800: 0.6%). The other assays’ linear ranges (CV) are: sGlu, 0–1190 mg/dL (0.8%); tCO$_2$, 10–40 mEq/L (3.5%); TP, 2.0–12.0 g/dL (1.8%); ALB (bromocresol green), 1.0–6.0 g/dL (1.8%). Gas panel measurements of $D_{Na}$, glucose (gGlu) and pH were performed on the ABL800 FLEX blood gas analyzer (Radiometer, Copenhagen) on heparinized whole blood samples collected using the safePICO sampler (Radiometer). The $D_{Na}$ assay is calibrated every 4 h, reports results as whole numbers and has a linear range (CV) of 7–350 mEq/L (0.9%). The other assays’ linear ranges (CV) are: gGlu, 0–1081 mg/dL (6%); pH, 6.3–8.0 (0.11%). The gas panel bicarbonate concentration (gHCO$_3$) is calculated with the Henderson–Hasselbalch equation.

**Data selection and pairing**

A data abstraction program searched the hospitals’ computerized laboratory database and retrospectively collected every metabolic and blood gas panel originating from a critical care unit during 2009–2011. Metabolic panels were excluded if they lacked $I_{Na}$, sGlu or ALB; hemolysis or lipemia was reported; or if sGlu exceeded 1081 mg/dL (the upper limit of the gGlu assay). Blood gas panels were excluded if they lacked $D_{Na}$ or gGlu. The abstracted data did not indicate whether the gas sample was arterial or venous.

If a gas panel and a metabolic panel of a given patient had been registered under 20 min apart, they were paired. This short time limit minimized the impact of ongoing therapy on serum sodium, and the chance that the latter member of a pair was collected specifically to confirm an abnormal sodium in the former, while allowing sufficient cases for estimate precision. No patient or panel was used twice. If a gas panel could be paired with two metabolic panels (or vice versa), the pair with the briefest time difference was selected; in case of a tie, the earlier pair was used. For each patient, the single pair with the briefest time difference was retained; in case of ties, the earliest pair was chosen. While review of the clinical events at the time of blood sampling for every pair was not feasible, we screened the pairs for unreliable data by comparing the four analytes shared by both panels (i.e. sodium, potassium, bicarbonate, glucose). If two or more shared analytes were markedly discordant, we examined the clinical context at the time of sampling, and excluded the pair if there was an extremely obvious clinical explanation for the discordance, i.e. (i) sampling during an acute event associated with rapid changes in water and cation balance [29, 30] or in glucose concentration [13] (e.g. during acute treatment of hypoglycemia, resuscitation for cardiac arrest or hemodialysis) or (ii) clear contamination of the serum sample by a fluid infusion distal to the phlebotomy site.

After exclusions, 774 paired panels remained; one pair lacked a TP value. The median registration time difference within each pair was 4 min (0–19 min). For sensitivity analyses, the pairs were divided into three subgroups based on the time difference: 0–1 min ($n = 169$); 2–5 min ($n = 283$); 6–19 min ($n = 322$).

**Statistical methods and data analysis**

Continuous variables are presented as the mean $\pm$ standard deviation (SD) and compared with the paired or unpaired t-test or ANOVA. Variances (SD$^2$) were compared by Levene’s test. Associations between continuous variables were tested with correlation or simple or multiple linear regression.
Regression coefficients are presented as the mean ± standard error (SE) or the mean and 95% confidence interval (95% CI).

The target of the analysis, ΔNa, was computed as INa minus DNa. Since INa and DNa were whole numbers, ΔNa values were integers. Mean ΔNa estimates the bias between INa and DNa assays. Inconsistency of the bias across the range of sodium values was tested by correlating ΔNa with the sum of INa and DNa [31]. ΔGlu was computed as gGlu minus fGlu. The expected associations of ΔNa with TP, ALB, ΔGlu, tCO2, and pH, and possible associations with chemistry analyzer and registration time difference were tested. Next, the trend of ΔNa was examined across TP and ALB, divided into categories, by ANCOVA with ΔNa as a covariate to adjust for regression to the mean [23]. As in prior studies [19, 21], the TP categories were made 1 g/dL wide, ~90% of the SD of TP. For consistency, the ALB category width was chosen to approximate 90% of the SD of ALB (i.e. 0.7 g/dL). Nonlinear effects were sought by modeling the categories with polynomial contrast, a method which simultaneously tests linear and nonlinear (quadratic and higher order) effects. If the effect of either ALB or TP was essentially linear, it was next treated as a continuous variable and its effect estimated by multiple linear regression, adjusted for DNa, ΔGlu, pH or tCO2, and other potential confounders. Modeling assumptions were tested, including residual analysis and testing for interactions. Statistical tests were performed using SPSS 17.0 (Chicago, SPSS Inc.).

**RESULTS**

Baseline data and simple comparisons

The INa assay was positively biased relative to the DNa assay (Supplementary data, Figure S1). The bias (mean ΔNa) was 2.3 ± 2.2 mEq/L overall (P < 10\(^{-12}\)), increasing slightly from 2.2 ± 2.4 mEq/L (n = 567) to 2.7 ± 1.7 mEq/L (n = 207) after the chemistry analyzer was changed from the Siemens A1650 to the A1800 (P < 10\(^{-3}\)); the variance of mean ΔNa declined with the change (P < 0.002), consistent with the imprecision data of the manufacturer. ΔNa did not significantly correlate with the sum of INa and DNa overall or in either analyzer subgroup. Mean ΔNa did not differ in three registration time subgroups. The mean values and ranges of all the analytes are listed in Supplementary data, Table S2. SGlu was incidentally noted to be negatively biased relative to gGlu (ΔGlu: −6.9 ± 18.0 mg/dL, P < 10\(^{-24}\)).

ΔNa showed inverse associations of a clinically relevant size with TP (ΔNa = 6.2 − 0.60 × TP, P < 10\(^{-17}\); Figure 1), ΔGlu (ΔNa = 2.1 − 0.026 × ΔGlu, P < 10\(^{-8}\)) and ALB (ΔNa = 3.8 − 0.41 × ALB, P < 10\(^{-3}\)). Modest direct effects on ΔNa were also found for both pH (ΔNa = −17.3 + 2.7 × pH, P < 0.01) and tCO2 (ΔNa = 1.4 + 0.036 × tCO2, P < 0.01). TCO2 also varied directly with both TP (tCO2 = 18.9 + 1.0 × TP, P < 10\(^{-6}\)) and ALB (tCO2 = 18.6 + 1.9 × ALB, P < 10\(^{-10}\)) raising the possibility of confounding. Neither TP nor ALB varied with pH.

**Multivariable analysis of ΔNa trend**

Mean ΔNa declined consistently across the range of TP values, divided into uniform categories (P < 10\(^{-14}\); Table 1). When TP category was modeled using polynomial contrast, only the linear component was significant (P < 10\(^{-11}\)). Mean ΔNa also declined across ALB categories (P < 10\(^{-7}\), Table 1), but far less uniformly, with little change evident in the range 2.0–4.1 g/dL, a pattern evident in three independent subgroups, defined by registration time (Supplementary data, Figure S2). Trend analysis indicated statistically significant linear (P < 10\(^{-4}\)) and cubic (P < 0.02) components to the effect of ALB category. Since TP was a much stronger and more

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**Table 1. Categorical analysis of the effects of TP and ALB on ΔNa**

<table>
<thead>
<tr>
<th>Category (g/dL)</th>
<th>Mean ± SD</th>
<th>N</th>
<th>ΔNa (mEq/L) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 3.9</td>
<td>3.4 ± 0.5</td>
<td>17</td>
<td>4.4 ± 2.2</td>
</tr>
<tr>
<td>4–4.9</td>
<td>4.5 ± 0.3</td>
<td>61</td>
<td>3.3 ± 2.0</td>
</tr>
<tr>
<td>5–5.9</td>
<td>5.5 ± 0.3</td>
<td>155</td>
<td>2.8 ± 2.0</td>
</tr>
<tr>
<td>6–6.9</td>
<td>6.5 ± 0.3</td>
<td>267</td>
<td>2.4 ± 2.3</td>
</tr>
<tr>
<td>7–7.9</td>
<td>7.4 ± 0.3</td>
<td>210</td>
<td>2.0 ± 2.1</td>
</tr>
<tr>
<td>8–8.9</td>
<td>8.3 ± 0.3</td>
<td>54</td>
<td>0.7 ± 2.4</td>
</tr>
<tr>
<td>≥ 9</td>
<td>9.2 ± 0.4</td>
<td>9</td>
<td>0.1 ± 1.1</td>
</tr>
</tbody>
</table>

**Table 1. Categorical analysis of the effects of TP and ALB on ΔNA**

<table>
<thead>
<tr>
<th>Category (g/dL)</th>
<th>Mean ± SD</th>
<th>N</th>
<th>ΔNa (mEq/L) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 1.9</td>
<td>1.7 ± 0.2</td>
<td>16</td>
<td>4.3 ± 2.6</td>
</tr>
<tr>
<td>2–2.6</td>
<td>2.4 ± 0.2</td>
<td>104</td>
<td>2.5 ± 2.2</td>
</tr>
<tr>
<td>2.7–3.3</td>
<td>3.0 ± 0.2</td>
<td>165</td>
<td>2.4 ± 2.1</td>
</tr>
<tr>
<td>3.4–4.1</td>
<td>3.8 ± 0.2</td>
<td>322</td>
<td>2.4 ± 2.3</td>
</tr>
<tr>
<td>4.2–4.8</td>
<td>4.4 ± 0.2</td>
<td>151</td>
<td>1.9 ± 2.2</td>
</tr>
<tr>
<td>≥ 4.9</td>
<td>5.1 ± 0.2</td>
<td>16</td>
<td>1.4 ± 1.9</td>
</tr>
</tbody>
</table>

*P < 10\(^{-14}\) by ANCOVA with DNa, as a covariate.

*P < 0.001 by ANCOVA with DNa, as a covariate.
Table 2. Effect of TP on ΔNa adjusted for glucose, tCO₂ and other covariates

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Coefficient ± SE</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP (per 1 g/dL)</td>
<td>−0.69 ± 0.06</td>
<td>−0.82 to −0.57</td>
<td>&lt;10⁻⁴⁴</td>
</tr>
<tr>
<td>ΔGLU (per mg/dL)</td>
<td>−0.026 ± 0.004</td>
<td>−0.034 to −0.019</td>
<td>&lt;10⁻¹⁰</td>
</tr>
<tr>
<td>tCO₂ (per mEq/L)</td>
<td>0.06 ± 0.01</td>
<td>0.04 to 0.08</td>
<td>&lt;10⁻⁵</td>
</tr>
<tr>
<td>ADVIA 1650</td>
<td>−0.42 ± 0.16</td>
<td>−0.10 to −0.74</td>
<td>0.01</td>
</tr>
<tr>
<td>(versus A1800)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔNa (per mEq/L)</td>
<td>−0.08 ± 0.01</td>
<td>−0.10 to −0.06</td>
<td>&lt;10⁻⁹</td>
</tr>
<tr>
<td>Constant</td>
<td>15.4 ± 1.7</td>
<td>12.0 to 18.8</td>
<td>&lt;10⁻¹⁷</td>
</tr>
</tbody>
</table>

*Coefficient and 95% CI in mEq/L.

ALB varied inversely with ΔNa, but not linearly (Table 1, Supplementary data, Figure S2), and trend analysis suggested the relationship was best described by a cubic model. Dimeski and Barnett [11] also found this relationship to be nonlinear. It might be expected that ALB would not correlate in a simple manner with the non-water fraction of serum in critically ill patients, in whom a fall in ALB tends to be offset by a reciprocal increase in acute-phase proteins [32]. We found that mean ΔNa declined consistently across TP categories (Table 2), similar to the categorical analysis by Dimeski et al. [21]. When we treated TP as a continuous variable, ΔNa decreased by 0.64 mEq/L (95% CI: 0.51–0.76 mEq/L) to 0.69 mEq/L (0.57–0.82 mEq/L) for each 1 g/dL increase in TP, depending on the covariates. The effect of TP was consistent in three independent registration time subgroups, and was also similar to the estimate of 0.7 mEq/L reported by Chow et al. [19]. Unlike prior studies, the present analysis also adjusted for the effects on ΔNa of pH or CO₂, the estimates of which were consistent with prior studies [8, 27, 28]. While some of the variability of the TP effect in prior studies might reflect the variety of chemistry and gas analyzers employed [33], we found it to be similar on two different chemistry analyzers, albeit from the same manufacturer (Figure 1). In any event, future studies should test a variety of different analytic platforms concurrently. When it is clinically important to attenuate the bias in ΔNa because the serum non-water fraction is abnormal or changing, a practical approach at present would be to subtract 0.7 mEq/L from ΔNa for each 1 g/dL fall in TP (or to add 0.7 mEq/L for each 1 g/dL increase in TP). For example, dilution of TP is predictable when crystalloids are infused intravenously to treat hyponatremia, or when water shifts out of cells in response to a rise in serum glucose. Correcting the resulting bias in ΔNa will improve accuracy in calculating the treatment rate of hyponatremia, and in estimating the fall in ΔNa to be expected when glucose rises [34]. Although the interchangeable use of ΔNa and ΔNa data has been discouraged [35], we suggest that it can be done by adjusting ΔNa to the value it would have at that TP concentration at which non-water bias is zero (TP₀).

Correcting for non-water bias

Correcting serum sodium for non-water bias 255

Discordance between results can result from measurement error, including random error (imprecision), calibration bias and interference specific to one assay. Selective interference with I₉Na, but not with D₉Na, is known to occur when the non-water fraction of serum deviates from its usual value of 7% [4, 6]. Theoretical and experimental work suggested that the difference between indirect and direct measurements of sodium from non-water bias should decrease by ~1 mEq/L for each 1 g/dL increase in TP [5, 6, 16]. Observational studies, however, have differed markedly in their estimates of the effects of TP and ALB on ΔNa. For example, a 1 g/dL increase in TP has been reported to have no effect on ΔNa [18], or to cause a linear decrease of 0.7, 1.2 or 1.4 mEq/L [17, 19, 20], or to have a nonlinear effect [11] (Supplementary data, Table S1). The exact source of this variation is unclear, as the studies differed in many ways, including the handling of several statistical issues, as reviewed earlier. The present study quantified the effects of TP and ALB on ΔNa, in a large retrospective database of critically ill patients, with testing for nonlinearity, avoidance of repeat measures, and adjustment for regression to the mean and other known influences on ΔNa.
samples were not necessarily simultaneous, some of the differences between $I_{Na}$ and $D_{Na}$ may be the result of a true change, resulting from changes in glucose concentration [13] or in cation and water balance [29, 30]. The regression models accounted for glucose, but not for water and cation balance; the resulting unexplained variance will tend to reduce the precision of the models. Another potential concern is selection bias because the data were retrospective. This bias should be minimal, however, because metabolic and gas panels are routinely obtained in every critical care patient in our institution, each such panel was captured by the software we employed and each patient was limited to one pairing. Generalizability may be a concern since the focus of our study, as in most prior studies, was critically ill patients, who also tend to be hypolipidemic. It is possible that TP may less closely represent the non-water fraction of serum in less seriously ill, normolipidemic, patients. However, as Sepulveda noted, the impact of TP on the non-water fraction tends to be much greater than that of lipids, owing to the wider absolute range of TP values observed in the clinical setting [40].

In summary, the water exclusion effect interferes with $I_{Na}$ measurements, causing a spurious difference to arise between $I_{Na}$ and $D_{Na}$. We found this difference increases linearly by ~0.7 mEq/L with each 1 g/dL decrease in TP, a ratio that may be clinically useful when correction of $I_{Na}$ for non-water bias is needed. But the exact TP value at which any given pair of $I_{Na}$ and $D_{Na}$ assays yields equivalent results is likely to vary by institution, implied by the finding, in prior work and our study, that $I_{Na}$ and $D_{Na}$ assays also differ by a constant or intercept bias, the value of which depends on the specific commercial assays being compared.

**SUPPLEMENTARY DATA**

Supplementary data are available online at http://ndt.oxfordjournals.org.

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**CONFLICTS OF INTEREST STATEMENT**

There was no specific financial support for the study. We acknowledge the use of the standard resources available to us as clinicians working for the Department of Veterans Affairs. Dr Goldwasser owns shares of common stock in two health-related companies with no obvious connection to this work (Merck, Johnson and Johnson), and Drs Ayoub and Barth have no financial conflicts of interest to disclose.

**REFERENCES**

Renal histologic changes and the outcome in patients with diabetic nephropathy

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ABSTRACT

Background. The progression of diabetic nephropathy (DN) is frequently determined by clinical parameters; however, the predictive value of histologic lesions remains largely unknown. Our aim was to evaluate the relationship between histologic changes and renal outcome in patients with type 2 diabetes mellitus (T2DM).

Methods. A total of 396 patients with T2DM and biopsy-proven DN who received follow-up for at least 1 year were recruited. The severity of different histologic lesions was assessed using the pathologic classification established by the Renal Pathology Society. Renal outcomes were defined by progression to end-stage renal disease and doubling of serum creatinine. The influence of histologic findings on renal outcomes was assessed using univariate and multivariate Cox regression.

Results. A univariate Cox regression showed that the severity of glomerular and interstitial lesions had a significant impact on renal outcomes (P < 0.001). Scores of vascular lesions demonstrated no association with renal outcomes (P > 0.05). A

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