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Graded interference with the direct potentiometric measurement of sodium by hemoglobin

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ABSTRACT

Objectives: Sodium concentration is measured by either indirect (I_{Na}) or direct potentiometry (D_{Na}), on chemistry and gas panels, respectively. A spurious difference between these methods ($\Delta Na = I_{Na} - D_{Na}$) can be confusing to the clinician. For example, variation in serum total protein (TP) is well known to selectively interfere with I_{Na}. Red cells have been suggested to interfere with D_{Na}, but both positive and negative interference have been reported. In this study, the effect of gas panel hemoglobin (Hb) on ΔNa was examined.

Methods: ΔNa was calculated in 772 pairs of closely-timed chemistry and gas panels (median: 4 min. apart), retrospectively collected from our critical care units, with 1 pair per patient. Hb was treated as a categorical or continuous variable and tested for linear and non-linear effects, with adjustment for 3 known influences on ΔNa—TP, bicarbonate (tCO_2), and the chemistry-gas panel glucose difference (ΔGlu).

Results: Hb ranged from 3.5 to 22.0 g/dL [35–220 g/L]. In categorical analysis, ΔNa increased with Hb, and the effect was essentially linear. By simple regression, ΔNa rose 0.06 ± 0.03[SE] mmol/L per 1 g/dL [10 g/L] increase in Hb ($p < 0.05$), but confounding was suspected because Hb also correlated ($p < 10^{-3}$) with TP, tCO_2, and ΔGlu. Using multiple regression to adjust for the confounders, ΔNa rose 0.15 ± 0.03 mmol/L per 1 g/dL [10 g/L] rise in Hb ($p < 10^{-6}$).

Conclusions: Increasing Hb spuriously decreases D_{Na} and increases ΔNa. A linear correction for this artifact can reduce the discordance between I_{Na} and D_{Na}, promoting their interchangeable use.

1. Introduction

Sodium concentration is commonly measured by the direct potentiometric method (D_{Na}) in blood gas panels, or by the indirect method (I_{Na}) in metabolic panels performed on automated chemistry analyzers. Since both measurements are used, a spurious difference between them ($\Delta Na = I_{Na} - D_{Na}$) may lead to confusion in the diagnosis and treatment of a dysnatremia [1]. Such a difference can arise from random error, calibration bias, or assay interference. For example, the water exclusion effect selectively interferes with I_{Na}. We recently derived a linear correction for that artifact in a large set of closely-timed pairs of chemistry and gas panels (median: 4 min apart) collected from a critical care setting, with total protein (TP) used to represent the water exclusion effect, adjusted for other effects on ΔNa, including glucose change, bicarbonate (or pH), and regression to the mean [2]. In that study, I_{Na} and D_{Na} were measured on the ADVIA Chemistry System (1650 or 1800) and the ABL800 Flex analyzers, respectively.

D_{Na} might be selectively affected by red cells. The red cell suspension effect is known to alter the liquid-junction potential [3], and comparisons of whole blood and plasma D_{Na} measurements suggest that red cells indeed interfere with D_{Na}, but both positive [4,5] and negative [6,7] interference have been reported. Greffe and Gouget found that direct measurements of another cation, lithium, tended to be depressed in a graded manner as sample hematocrit was increased [8]. Using the data collected in our prior study, we examined the effect of variation of hemoglobin (Hb) on ΔNa, adjusting for the other known effects on ΔNa.

2. Methods

2.1. Data collection and pairing

A data extraction program was used to collect every serum metabolic panel and whole blood gas panel originating in the critical care units of the Veterans Affairs New York Harbor Healthcare System during 2009–2011. Each metabolic panel was paired with the gas panel closest

Abbreviations: I_{Na}, Sodium measured by indirect potentiometry; D_{Na}, Sodium measured by direct potentiometry; ΔNa, Indirect minus direct potentiometric measurements of sodium; TP, Total protein; Hb, Hemoglobin; tCO_2, Total carbon dioxide; sGlu, Serum glucose in chemistry panel; gGlu, Plasma glucose in blood gas panel; ΔGlu, Chemistry panel glucose minus gas panel glucose; SE, Standard error; CI, Confidence interval.

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to it in time. Metabolic panels were excluded if they lacked values for I_{Na}, TP, total carbon dioxide (tCO_2), or glucose (sGlu), if they had been received by the laboratory 20 min or more apart from the closest-in-time gas panel, or if hemolysis or lipemia had been noted. Gas panels were excluded if they lacked values for D_{Na}, Hb, or glucose (gGlu). Other exclusion criteria are described in our prior report [2]. Limiting each patient to the pair with the briefest elapsed time left 772 pairs, with a median intra-pair time gap of 4 min (0–19 min.). The protocol was approved by the institutional review board.

2.2. Analytic platforms

Serum metabolic panels were performed on the *ADVIA Chemistry System 1650* analyzer from 2009 through early 2011 (n = 565), or the *ADVIA 1800* thereafter (n = 207) (Siemens Healthcare Diagnostics, Tarrytown, NY). Gas panels were performed using the *ABL800 FLEX* blood gas analyzer (Radiometer, Copenhagen) on samples collected using the safePICO sampler (Radiometer). The gas panel Hb assay has a linear range of 0.0–27.7 g/dL and a coefficient of variation of 2.7%. The characteristics of the other assays are described in our prior report [2].

2.3. Data analysis

The intra-pair differences were calculated for sodium (ΔNa) and glucose (ΔGlu = sGlu − gGlu). Continuous variables were summarized as mean ± standard error of the mean (SE) or the mean and 95% confidence interval (CI), and compared using paired and unpaired *t*-tests and simple correlation. ΔNa was initially explored using the approach of Bland and Altman [9]. Next, we examined the effect of Hb—divided into 7 categories spaced approximately 2 g/dL [20 g/L] apart—upon ΔNa using ANCOVA with polynomial contrast to test for linear and non-linear effects, and with testing of model assumptions including of homogeneity of covariates. If the Hb effect was shown to be essentially linear, it was next estimated by simple and multiple linear regression. Covariates included in multivariate models were: TP, ΔGlu, and tCO_2, three known effects on ΔNa [2]; chemistry analyzer; and D_{Na}, which adjusts for regression to the mean [10].

In one sensitivity analysis, the patients were divided into three subgroups based on the time difference between metabolic and gas panels: 0–2 min. (n = 239); 3–6 min. (n = 256); 7–19 min. (n = 277). In another sensitivity analysis, limited to the subgroup of patients who had complete blood panels (CBC) collected simultaneously with their metabolic panels (n = 711), CBC-derived Hb was used for the analysis instead of gas panel Hb. SPSS 17.0.2 software was used (Chicago, IL).

3. Results

The mean and range of each variable are listed in Supplemental Table 1. The I_{Na} assay was positively biased relative to the D_{Na} assay (mean ΔNa: 2.3 ± 0.1 mmol/L, $p < 10^{-124}$). The bias rose slightly after the chemistry analyzer was changed in 2011 (Siemens A1650: 2.2 ± 0.1 mmol/L; A1800: 2.7 ± 0.1 mmol/L; $p < 10^{-3}$). ΔNa was distributed normally (Supplemental Fig. 1), and did not correlate with the sum of I_{Na} and D_{Na} overall or in either analyzer subgroup.

Hb ranged from 3.5–22.0 g/dL [35–220 g/L] (mean: 11.4 ± 0.1 g/dL [114 ± 1 g/L]). From the lowest Hb category (mean Hb: 5 g/dL [50 g/L]) to the highest (mean Hb: 17 g/dL [170 g/L]), mean ΔNa rose 1.2 mmol/L (Table 1); modeling Hb with polynomial contrast showed that only the linear trend was significant (P < 0.02). By simple linear regression, ΔNa rose 0.06 ± 0.03 mmol/L per 1 g/dL [10 g/L] rise in Hb ($p < 0.05$; Supplemental Fig. 2). However, that estimate of the Hb effect is likely biased by confounding, because Hb also correlated with TP ($p < 10^{-27}$), tCO_2 ($p < 10^{-7}$), and ΔGlu ($p < 10^{-3}$), each of which influenced ΔNa (ΔNa vs TP, $p < 10^{-16}$; ΔNa vs tCO_2, $p < 10^{-2}$; ΔNa vs ΔGlu, $p < 10^{-7}$)).

Table 1
Mean (±SE) ΔNa (mmol/L) by Hb category.

Hb Category[a]	N	Mean Hb	ΔNa, Unadjusted[b]	ΔNa, Adjusted[c]
<6 g/dL	17	5.0	1.4 ± 0.4	1.3 ± 0.5
6–7.9 g/dL	43	7.2	1.8 ± 0.3	1.5 ± 0.3
8–9.9 g/dL	166	9.1	2.3 ± 0.2	2.0 ± 0.2
10–11.9 g/dL	240	11.0	2.4 ± 0.1	2.4 ± 0.1
12–13.9 g/dL	184	12.9	2.4 ± 0.2	2.5 ± 0.1
14–15.9 g/dL	83	14.8	2.5 ± 0.3	2.8 ± 0.2
≥16 g/dL	39	17.0	2.6 ± 0.3	3.1 ± 0.3

Abbreviations: ΔNa: indirect minus direct potentiometric measurements of sodium; Hb, hemoglobin; TP, total protein; ΔGlu, chemistry panel glucose minus gas panel glucose; tCO2, total carbon dioxide; D_{Na}, direct potentiometric measurement of sodium.
[a] To convert Hb from g/dL to g/L, multiply by 10.
[b] P < 0.02 for linear trend.
[c] $P < 10^{-4}$ for linear trend. Means are adjusted for TP, tCO_2, ΔGlu, analyzer, and D_{Na}, all set to their mean values.

The effect of Hb category became more evident in a full model that adjusted for the confounders and other pre-specified covariates, with mean ΔNa rising 1.8 mmol/L from the lowest Hb category to the highest (Table 1); again, only the linear trend was significant ($p < 10^{-4}$). By multiple linear regression (Table 2), ΔNa rose 0.15 mmol/L per 1 g/dL [10 g/L] increase in Hb (95% CI: 0.09–0.21 mmol/L; $p < 10^{-6}$). In two sensitivity analyses, the Hb effect was similar in 3 independent time subgroups (0–2 min.: 0.17 ± 0.05 mmol/L; 3–6 min.: 0.12 ± 0.05 mmol/L; 7–19 min.: 0.17 ± 0.06 mmol/L), and the effect was also essentially unchanged when CBC-derived Hb was used instead of gas panel Hb (Supplemental Table 2).

4. Discussion

Errors in the measurement of serum sodium can adversely affect patient care. In a prior study, we examined the discordance between I_{Na} and D_{Na} measurements made in a large critical care cohort with the ADVIA (1650 and 1800) and ABL800 analyzers, respectively, and found both a variable bias, with each 1 g/dL [10 g/L] increase in TP resulting in a 0.7 mmol/L mean decrease in I_{Na}, as well as a constant, calibration bias, with I_{Na} being 2.3 mmol/L higher than D_{Na} on average [2]. As a result of these effects, the I_{Na} and D_{Na} assays we studied tended be in closest agreement only when TP reached the unusually high value of 10 g/dL [100 g/L]; since calibration bias tends to vary among instruments, it is unsurprising that the specific TP value at which I_{Na} and D_{Na} assay pairs studied by others most closely agreed has varied from 5.0 to 10.3 g/dL [50–103 g/L] [2,11]. In the present study of the same cohort, we found a novel variable bias caused by Hb. Each 1 g/dL [10 g/L] increase in Hb independently decreased D_{Na} by approximately 0.15 mmol/L relative to I_{Na}. Although this estimate remained consistent in sensitivity analyses, its confirmation is obviously necessary, with the

Table 2
Multiple linear regression model of ΔNa (mmol/L)[a].

Predictors	Coefficient (±SE)[b]	P
(Constant)	14.3 ± 1.7	$<10^{-15}$
Hb (per g/dL)	0.15 ± 0.03	$<10^{-6}$
TP (per g/dL)	−0.80 ± 0.07	$<10^{-29}$
ΔGlu (per mg/dL)	−0.024 ± 0.004	$<10^{-8}$
tCO_2 (per mmol/L)	−0.05 ± 0.01	$<10^{-4}$
D_{Na} (per mmol/L)	−0.08 ± 0.01	$<10^{-9}$
ADVIA 1650 (vs 1800)	−0.46 ± 0.16	<0.004

Abbreviations: ΔNa: indirect minus direct potentiometric measurements of sodium; Hb, hemoglobin; TP, total protein; ΔGlu, chemistry panel glucose minus gas panel glucose; tCO2, total carbon dioxide; D_{Na}, direct potentiometric measurement of sodium.
[a] Model adjusted $R^2 = 0.24$.
[b] To convert coefficients of Hb and TP from mmol/L increase ΔNa in per g/dL increase in predictor to mmol/L per g/L increase, multiply the coefficients by 0.1. To convert the coefficient of ΔGlu from mmol/L per mg/dL increase in predictor to mmol/L per mmol/L increase, multiply the coefficient by 18.

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