Effect of low serum total protein on sodium and potassium measurement by ion-selective electrodes in critically ill patients

E. CHOW*, N. FOX*, and R. GAMA*,†

*Department Clinical Chemistry, New Cross Hospital, Wolverhampton, West Midlands; and †Research Institute, Healthcare Sciences, Wolverhampton University, Wolverhampton, West Midlands, UK

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ABSTRACT

Hypoproteinaemia may lead to spuriously high electrolyte values using indirect ion-selective electrodes (ISE) compared to direct ISEs. This study evaluates the impact on electrolyte status assessment of direct compared to indirect ISE sodium and potassium measurements in samples from critically ill patients who have a high prevalence of hypoproteinaemia. Serum sodium and potassium measurements were compared using indirect and direct ISE in 190 samples received from critical care units over a three-week period. Serum sodium and potassium measurements were higher ($P<0.0001$) using indirect ISE (140.0±5.0 and 4.5±0.6, respectively) compared to direct ISE (136.5±5.2 and 4.5±0.6, respectively). The calculated difference between indirect and direct ISE values for sodium increased as total protein concentration decreased (Y=7.2–0.07X, 95% CI slope –0.1 to –0.05, $P<0.0001$, $r^2=0.14$). Hypoproteinaemia was present in 85% of samples. Indirect ISE, compared to direct ISE, misclassified 28% of samples as pseudonormonatraemia (19%), pseudohypernatraemia (8%), pseudonormokalaemia (0.8%) and pseudohyperkalaemia (0.4%). Hypoproteinaemia is common in critically ill patients and this may lead to spuriously high indirect ISE electrolyte measurements, resulting in significant misclassification of electrolyte (particularly sodium) status. In such patients, direct ISE (as employed in point-of-care testing) offers more accurate and consistent electrolyte results than does indirect ISE (commonly used in major laboratory analysers).

KEY WORDS: Electrodes, ion selective.
Hyponatremia.
Hypoproteinemia.
Point-of-care testing.
Pseudohypernatremia.
Pseudonormonatremia.
Sodium.

Introduction

Direct ion selective electrodes (ISE) measure the activity of ions in water, which is directly proportional to their concentration. Indirect ISE, however, measures activity of ions in a diluted sample. Ion activity, and hence concentration, is then related to total sample volume rather than to water volume. Within this lies the assumption that plasma consists of 93% water and 7% dissolved solids.

It is well recognised that an increase in dissolved solids, as occurs in hyperproteinaemia, reduces the water fraction of plasma, and thus the indirect ISE measurement of sodium results in pseudohyponatraemia.[1,2] Less well recognised is that an increase in the plasma water fraction, as occurs in hypoproteinaemia, may result in pseudonormonatraemia and pseudohypernatraemia when sodium is measured by indirect ISE.[3,4]

In critically ill patients, the early recognition and treatment of hyponatraemia is important in order to prevent neurological damage.[5–8] Hypoproteinaemia may be relatively common in acutely ill patients[9] and this may mask genuine hyponatraemia if electrolytes are measured by indirect ISE. Indeed, it has been reported that direct ISE sodium concentrations are lower than those measured by indirect ISE in critically ill patients.[10,11]

Thus, this study investigates the prevalence of hypoproteinaemia in samples from critically ill patients and measures serum sodium and potassium concentrations using indirect and direct ISE in these samples to evaluate whether or not these have any impact on the classification of electrolyte status. The study also investigates the relationship between calculated (indirect–direct ISE) electrolyte differences and serum total protein concentrations.

Correspondence to: Professor Rousseau Gama
Department of Clinical Chemistry, New Cross Hospital, Wolverhampton, West Midlands WV10 0QP
Email: rousseau.gama@rwh-tr.nhs.uk

Materials and methods

All blood samples received over a three-week period from critical care units at New Cross Hospital, Wolverhampton, UK, were included in this service evaluation, comparing sodium and potassium measurement using direct and indirect ISEs. Blood collected into a gel tube (Sarstedt Safety Monovette serum S/4.7, Aktiengesellschaft, Germany) was allowed to clot and the serum was separated for analysis. Following anonymisation, serum sodium and potassium

concentrations were analysed by indirect ISE on the Roche Modular ISE 900 (Roche Diagnostics, Mannheim, Germany) and direct ISE on the Roche AVL9181 electrolyte analyser (Roche Diagnostics). Samples with insufficient volume for electrolyte measurement on both analysers were excluded, as were those with visible lipaemia. Thirty randomly selected normoproteinaemic serum samples with normal biochemistry were used as controls.

For the Roche Modular ISE, intra-assay and inter-assay coefficients of variation (CV) for serum sodium were both <1.0% and for serum potassium were 0.34% and 1.6%, respectively. For the AVL9181 analyser, intra-assay and inter-assay CVs for serum sodium were both <1.0% and for serum potassium were <1.5% and 2.6%, respectively. Serum total protein was measured using the biuret method on the Roche Modular automated analyser (Roche Diagnostics). Respective inter-assay and intra-assay CVs for serum total protein were 0.6% and 1.0%, respectively.

Reference intervals used to define serum abnormalities were 135–145 mmol/L sodium, 3.5–5.0 mmol/L potassium and 60–80 g/L total protein.

Data were normally distributed. Therefore, results are expressed as mean ± standard deviation (SD). Bland Altman analysis and two-tailed paired *t*-tests were used to assess the significance of differences between paired variables. Unpaired *t*-test and ANOVA were used to compare the significance of differences between variables between two groups or three or more groups, respectively. Linear regression analysis was used to determine the significance of the relationship between variables. Statistical analyses were performed using GraphPad Prism Version 5 software (GraphPad Software, San Diego, USA). $P<0.05$ was considered statistically significant.

Results

During the three-week study period, 198 samples were received from the intensive care and cardiothoracic critical care units. Eight samples were excluded because of inadequate sample volume, leaving 190 samples for further analysis. Data on electrolyte and protein analyses on samples from critically ill patients and controls are shown in Table 1.

Table 1. Plasma electrolytes and proteins in the study and control groups.

	Critically ill group	Control group
Number	190	30
Indirect ISE Na^+ (mmol/L)	140.0±5.0*	141±3.2*
Direct ISE Na^+ (mmol/L)	136.5±5.2*	139.6±3.5*
Indirect–direct ISE Na^+ (mmol/L)	3.5±2.0†	1.4±1.6†
Indirect ISE K^+ (mmol/L)	4.5±0.6*	4.6±0.7*
Direct ISE K^+ (mmol/L)	4.5±0.6*	4.6±0.6*
Indirect–direct ISE K^+ (mmol/L)	0.03 ± 0.1	0.01±0.07
Total Protein (g/L)	50.5±10.6	69.3±4.7

*$P<0.0001$ for direct compared to indirect ISE measurements.

†$P<0.0001$ for critically ill patients compared to controls.

Results presented as mean±SD.

Table 2. Calculated difference for sodium and potassium between indirect and direct ISE values for samples with different total protein levels.

Total protein (g/L)	Indirect–direct ISE Na^+ (mmol/L)	Indirect–direct ISE K^+ (mmol/L)
Samples from critically ill patients (n=190)		
<40 (n=25)	4.72±1.88‡,§,¶	0.10±0.11‡,§,¶
40–49 (n=68)	3.87±1.84§,¶	0.04±0.09§
50–59 (n=69)	3.24±1.99*,†,§,¶	0.01±0.10*
60–80 (n=28)	1.89±1.73*,†,‡	-0.02±0.10*
Control samples (n=30)		
60–80	1.40±1.60*,†,‡	0.01±0.07*

*$P<0.05$ compared to total protein <40 g/L

†$P<0.05$ compared to total protein 40–49 g/L

‡$P<0.05$ compared to total protein 50–59 g/L

§$P<0.05$ compared to total protein 60–80 g/L

¶$P<0.05$ compared to controls (total protein 60–80 g/L)

Results presented as mean±SD.

In summary, hypoproteinaemia (serum total protein <60 g/L) was present in 85% of samples from critically ill patients. Serum sodium and potassium concentrations were higher ($P<0.0001$) when measured by indirect ISE compared to direct ISE in both the critically ill and control groups. The calculated difference between indirect and direct ISE electrolyte values was higher ($P<0.0001$) for sodium but similar ($P=0.22$) for potassium in critically ill patients compared to controls.

In samples from critically ill patients, Bland Altman analysis of (indirect–direct) ISE values for sodium and potassium had 95% limits of agreement between –0.52 and 7.45 mmol/L and –0.18 and 0.24 mmol/L, respectively (Fig. 1).

There was a linear relationship between (indirect–direct) ISE electrolyte values and total protein concentrations (Fig. 2). As total protein concentrations decreased, there was an increase in (indirect–direct) ISE values for sodium ($Y=7.2–0.07X$, 95% CI slope –0.1 to –0.05, $P<0.0001$, $r^2 = 0.14$) and potassium ($Y = 0.2 – 0.003\ X$, 95% CI of slope –0.004 to –0.002, $P<0.0001$, $r^2=0.09$).

The (indirect–direct) ISE electrolyte values stratified according to total protein concentration are shown in Table 2. Compared to the controls (total protein 60–80 g/L), the (indirect–direct) ISE sodium and potassium values were similar in normoproteinaemic (total protein 60–80 g/L) but higher ($P<0.05$) in hypoproteinaemic (total protein <60 g/L) critically ill patients.

Indirect ISE, compared to direct ISE, misclassified 28% of the 190 samples as pseudonormonatraemia (19%), pseudohypernatraemia (8%), pseudonormokalaemia (0.8%) and pseudohyperkalaemia (0.4%).

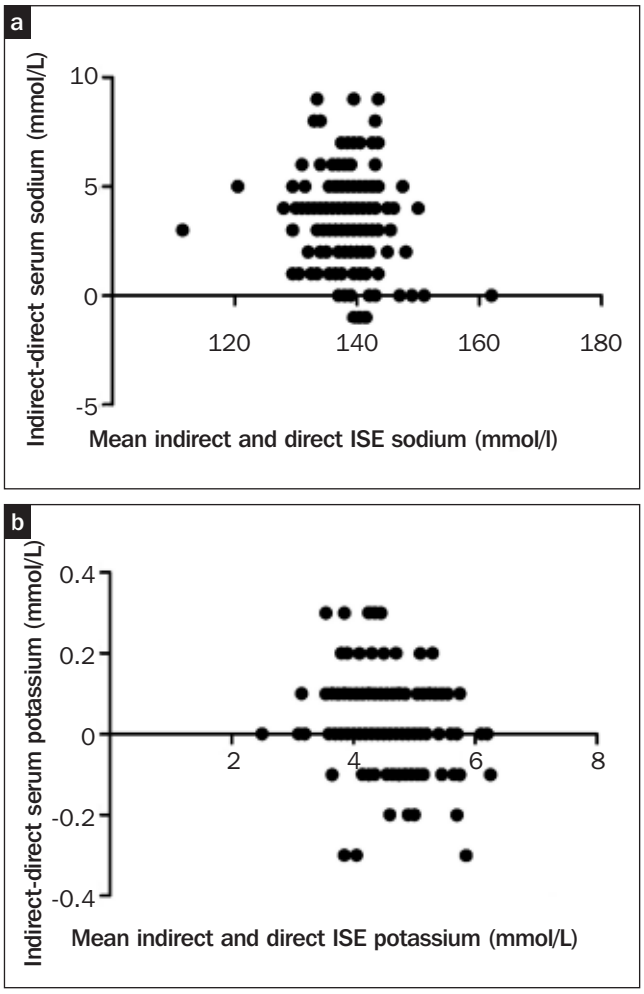

Fig. 1. Bland Altman analysis of the calculated difference between indirect and direct ISE measurements for a) sodium and b) potassium.

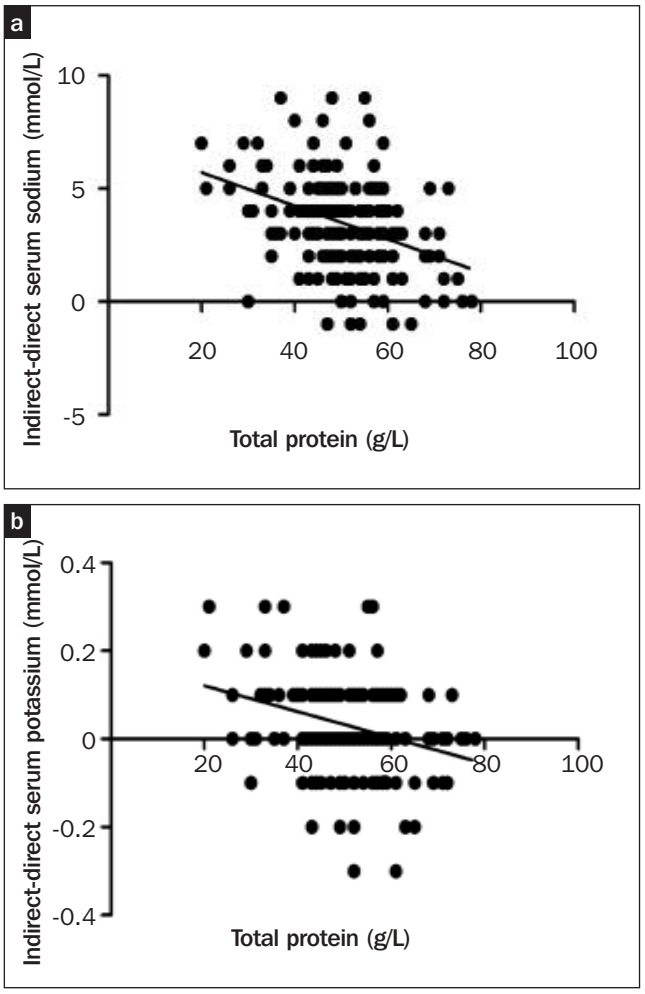

Fig. 2. Regression analysis of total protein and calculated difference between indirect and direct ISE for a) sodium and b) potassium.

Discussion

The higher indirect ISE than direct ISE values for sodium and potassium in samples collected from critically ill patients are consistent with those reported previously.[10,11] These retrospective studies, however, compared point-of-care testing (POCT) to central laboratory testing. In consequence, they compared electrolyte measurements in different types of sample (whole blood and plasma or serum) and collection tubes (heparinsed blood-gas syringes and lithium-heparin or clot-activating gel tubes), and these factors may affect electrolyte measurement.[12] They also failed to consider the contribution, if any, of method bias to the difference between indirect and direct electrolyte results.

The present study demonstrated higher indirect ISE than direct ISE serum electrolyte activities in the same sample, which suggests that the difference between indirect and direct ISE electrolyte values is independent of sample type and collection tube. Although the indirect and direct ISEs in this study were calibrated according to respective manufacturer instructions, and performance was verified by internal quality control and external quality assurance, data from control samples indicated a small positive bias towards the direct ISE methodology. However, this bias does not fully explain the greater calculated difference between indirect and direct electrolyte values in the critically ill patients compared to controls.

The increased calculated (indirect–direct) electrolyte difference in critically ill patients is likely to be a protein effect, as first it was similar in the normoproteinaemic control samples and in the normoproteinaemic samples from critically ill patients, and, second, calculated electrolyte differences increased as serum total protein concentration decreased.

The high prevalence of hypoproteinaemia in samples received from critically ill patients in this study and others[9] makes indirect ISE electrolyte measurement a potentially significant clinical problem for the assessment of sodium but less so for potassium. In this study, indirect ISE, compared to direct ISE, misclassified 28% of samples largely as pseudonormonatraemia (19%) and pseudohypernatraemia (8%), with very few misclassified as pseudonormokalaemia (0.8%) and pseudohyperkalaemia (0.4%).

Unrecognised hyponatraemia and pseudohypernatraemia may lead to inappropriate fluid therapy. This may have adverse effects as critically ill patients are prone to hyponatraemic encephalopathy.[5] Accurate regular electrolyte analyses are required to monitor the emergency correction of hyponatraemia to avoid central pontine myelinolysis.[6,13] Thus, it is important for clinical and

laboratory staff to be aware of the limitations of methods employed for sodium and potassium analyses, and any clinical decisions should be based on an accurate and reliable method.

Direct ISE reflects plasma electrolyte activity more accurately than does indirect ISE, but most central laboratory analysers use indirect ISE because of its smaller volume requirement. However, the results presented here support the International Federation of Clinical Chemistry and Laboratory Medicine recommendation that, in specimens with abnormal total protein concentration, measurement of electrolytes in undiluted samples will reflect sodium activity more accurately.[14] Therefore, direct ISE should be used to measure electrolytes not only in hyperproteinaemic samples but also in hypoproteinaemic samples.

As hypoproteinaemia is common in critically ill patients, the authors of the present study suggest that direct ISE should be used to measure electrolytes in samples in this group of patients. Point-of-care testing equipment utilises direct ISE technology whereas major laboratory analysers utilise indirect ISE. Therefore, clinicians and laboratory staff should be aware that POCT analysers offer the more accurate and consistent results necessary for the assessment, management and monitoring of electrolyte status in critically ill patients. In hypoproteinaemic samples received in the laboratory, direct ISE electrolyte measurement should be considered in those patients who have an indirect ISE serum sodium level <140 mmol/L or >145 mmol/L, in order to minimise electrolyte misclassification. □

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