Letter to the Editor

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Disagreement between direct and indirect potentiometric Na^+ determination in infancy and childhood

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To the Editor,

The blood Na^+ concentration is currently determined by either indirect- or direct-reading potentiometry [1–3]. Indirect potentiometry involves presentation of a pre-diluted plasma sample to the measuring electrode [1]. By contrast, direct potentiometry involves presentation of an undiluted whole-blood sample to the electrode [1]. As both measurements are used, a difference between them may lead to confusion in the interpretation of (dys)natremia [1–3]. The direct and the indirect methods have been recently compared in adults and in newborns [4–6]. The current analysis was performed to characterize the disagreement between direct and indirect potentiometric Na^+ measurements in infants and children.

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A data abstraction program was used to search and retrospectively collect every metabolic panel and blood gas panel originating from venous blood taken in patients ≥4 weeks to ≤18 years of age managed at the Pediatric Emergency Department of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, between January and December 2018. Metabolic panels were included exclusively if they did not lack hemoglobin, white blood cell count, C-reactive protein, creatinine, urea, albumin, and indirect Na^+, and blood gas panels if they did not lack direct Na^+. The extracted data also included information on the main underlying clinical condition.

The metabolic panel was performed using a Sysmex blood cell counter XN-9000™ for hemoglobin and white cell count and a Roche Cobas 8000 c702 analyzer for plasma C-reactive protein (immunoturbidimetry), creatinine (Jaffe colorimetry), urea (urease assay), albumin (bromocresol green colorimetry), and Na^+ (indirect potentiometry). The blood gas panel was performed using a GEM® Premier™ 4000 analyzer for whole-blood Na^+ (direct potentiometry).

Normality of continuous variables was tested using the D'Agostino-Pearson omnibus test. Continuous variables are expressed as mean and standard deviation or median and interquartile range, as appropriate. Agreement between direct and indirect sodium measurements was evaluated using the Bland-Altman-Wickham method and its 95% limits of agreement. In addition, Cohen's kappa coefficient was calculated to take into account the possibility of agreement occurring by chance. The two-tailed t-test for two independent samples was used to assess the presence of potential differences between values obtained from the two methods.

To further investigate potential determinants of disagreement between the two methods, we fitted a series of ordinary least-squares multiple regression models including the difference between methods (direct – indirect measurement) as the dependent variable and the following independent variables: age, sex, hemoglobin, white

blood cell count, C-reactive protein, creatinine, urea, and albumin. Models including clinically meaningful pairwise interaction terms were also considered. Model selection was carried out combining results from likelihood ratio tests on single parameters and the Akaike Information Criterion. Significance was assumed when $p < 0.05$. The study was approved by the Institutional Review Board and performed in accordance with the principles of the Declaration of Helsinki.

A total of 1373 patients were included, as shown in Table 1. Mean sodium level was lower ($p < 0.00001$) when measured by the direct (134.3 ± 3.6 mmol/L) as compared with the indirect (137.7 ± 3.1 mmol/L) method. The Bland-Altman-Wickham plot (Figure 1) showed a mean difference between the two methods of –3.4 with wide limits of agreement (95% limits of agreement, –8.3 to 1.6) mmol/L. Cohen's kappa statistics was equal to 33.2%, indicating fair agreement between the two methods.

Table 2 shows the results from the multiple regression analysis. After model selection based on clinical plausibility and the Akaike Information Criterion, age, hemoglobin, and the pairwise interaction terms between age and creatinine, age and albumin, and albumin and hemoglobin were correlated with the difference between the two methods.

The present analysis points out that the difference between blood sodium estimates returned by direct and indirect potentiometry is relevant also in infants and children. The difference is related especially to age and hemoglobin. Infants have rather low levels of immunoglobulins as a result of the maternal immunoglobulin falling and the infant's ones just starting to be made [7]. Similarly, the concentration of other circulating pools, such as clotting and anti-clotting factors and lipids, is lower in infancy [8, 9]. Measurement of sodium by ion-selective electrodes in undiluted samples is unaffected by modifications in

Table 1: Clinical and laboratory characteristics of 1373 patients ≥4 weeks to ≤18 years of age included in the present report.

N	1373
Males/females, n	773/600
Age	
Years	2.7 [0.96–7.0]
≥4 weeks to ≤12 months, n	349
1–18 years, n	1024
Main initial diagnosis	
Abdominal condition, n	437
Lower respiratory illness, n	381
Upper respiratory illness, n	206
Central nervous system condition, n	111
Fever, n	111
Trauma, n	27
Genitourinary condition, n	12
Further conditions, n	88
Hemoglobin, g/L	121 ± 15.3
White blood cell count, 10^9/L	12.0 ± 5.9
Plasma level	
C-reactive protein, mg/L	3.8 [2.9–19]
Creatinine, µmol/L	34 [26–44]
Urea, mmol/L	3.8 [2.8–5.2]
Albumin, g/L	44 ± 4.6

Data are presented as relative frequency, median with interquartile range within brackets, or mean ± SD.

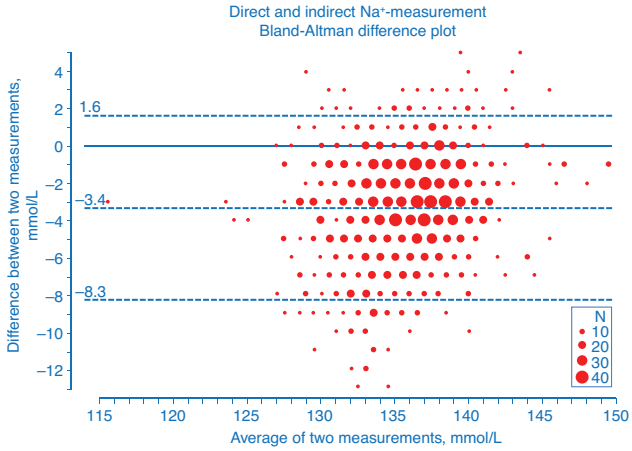

Figure 1: Bland-Altman-Wickham plot comparing the two measurement techniques.
This presentation strategy draws bigger dots if there is more than one data point at a given location. The mean difference is indicated by the center dashed line. The 95% limits of agreement are indicated by the upper and lower dashed lines.

Table 2: Ordinary least-squares regression models: results from models including main effects and pairwise interaction terms (n = 1373).

Variables	β-Coefficient	Standard error	p-Value
Intercept	4.90	4.55	0.282
Age	0.63	0.19	0.001[b]
Gender	–0.01	0.14	0.934
Albumin	–0.19	0.10	0.073
Hemoglobin	–0.10	0.04	0.011[a]
Urea	0.06	0.05	0.182
Creatinine	0.01	0.01	0.105
C-reactive protein	0.00	0.00	0.260
White blood cell count	0.00	0.01	0.930
Age:creatinine	0.00	0.00	0.034[a]
Urea:creatinine	0.00	0.00	0.100
C-reactive protein:white blood cell count	0.00	0.00	0.184
Age:albumin	–0.01	0.00	0.042[a]
Age:hemoglobin	0.00	0.00	0.309
Albumin:hemoglobin	0.00	0.00	0.020[a]

[a]$p < 0.05$, [b]$p < 0.01$.

the circulating non-water fraction. Hence, we speculate that the changing composition of mentioned fraction accounts, at least in part, for the observed effect of age on the disagreement between direct and indirect Na^+ measurements. The significant association between hemoglobin and difference between direct and indirect measurements observed in our study confirms the results of a recent study [4]. It has been postulated that hemoglobin might interfere with the membrane electrode when Na^+ is determined in whole blood by direct potentiometry. We also found an association with the following pairwise interaction terms: age and creatinine, age and albumin, and finally hemoglobin and albumin. However, as albumin and creatinine vary with age [10], this analysis is not able to verify if albumin and creatinine play a direct or an indirect role in the mentioned associations.

It is important to consider some strengths and limitations of this study. Its major strength is that it involved more than 1300 infants and children. Furthermore, the same blood sample was used for both Na^+ measurements. The main limitation relies on the retrospective study design, which does not allow to assess the reproducibility of the measurements. Finally, available data do not allow to accurately estimate the non-water fraction of plasma that might be severely altered in some emergency conditions.

In conclusion, the present data point out that the direct technology should be preferred for Na^+ measurement also in infants and children. To prevent possible interferences between hemoglobin and electrode membrane, we speculate that for the future the determination of Na^+ by direct potentiometry is assessed in plasma instead of whole blood.

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