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Estimating blood nitrate concentration from interference on radiometer ABL800 chloride electrode

To the Editor,

Nitrate species reportedly interfere with blood chloride measurement by ion selective electrodes (ISEs), which are commonly used to measure chloride in clinical laboratories. This phenomenon has been described with multiple instruments, including ABL700 and ABL800 (Radiometer Medical, Denmark),^{1,2} and Cobas Integra (Roche Diagnostics, USA).³ There is evidence that nitrate anions do not interfere with chloride measurements on Ci8200 Architect (Abbott Diagnostics, USA). The relationship between plasma nitrate concentration and changes in chloride readings is positive and linear. Plasma nitrate concentrations can become significant after ingestion of sodium nitrate or from the use of topical creams containing cerium nitrate.¹

Historically, nitrite (NO_2^-) and nitrate (NO_3^-) poisonings have been rare occurrences, with few published reports. However, in recent years a number of online sources have promoted sodium nitrite ingestion as a means of committing suicide, including Exit International. As a result, the number of presentations to emergency departments of nitrite poisonings (and nitrate poisonings) are expected to increase. One case report of nitrate ingestion describes a patient who presented with gastrointestinal symptoms and remained haemodynamically stable during his admission.⁴ Approximately 5% of ingested nitrate is converted to nitrite by flora of the alimentary canal.⁴ It is the absorbed nitrite species which are believed to be responsible for the life-threatening methaemoglobinaemia in cases of nitrate poisonings.⁴

Nitrate measurement is not routinely performed in clinical laboratories, however an estimation of a patient's blood nitrate could be made from discordance in chloride results between instruments that are susceptible to nitrate

interference (e.g., ABL800, Radiometer Medical) and those that are not (e.g., Alinity, Abbott Diagnostics). The disparity in susceptibility to nitrate interference can be explained by fundamental differences in electrode design. The Abbott instruments, including the Alinity, use a silver nitrate redox system to measure chloride, whereas the Radiometer instruments, including the ABL800, use an ion-selective polymer membrane.

Solid state silver will react with chloride anions to produce a measurable voltage, but will not react with nitrate because of its greater solubility. On the other hand, polymer membranes contain ion exchange molecules, often quaternary ammonium, which reside in channels of resin and could ferry nitrate as well as chloride from one side of the membrane to the other. Nitrate species have a high affinity for quaternary ammonium compounds due to their highly lipophilic nature. The aim of this study was to use a linear regression model to develop an equation that could be used in the acute care setting to estimate a patient's plasma nitrate concentration based on differences in chloride readings between Abbott and Radiometer instruments.

Nitrate-spiked patient samples were run by both Alinity and ABL800 methods. Three dilution series were produced, each comprising pooled plasma from a different random patient. Each dilution series consisted of five nitrate-spiked samples as well as a negative control. The volume of nitrate added to each sample was $\leq 1\%$. In detail, six 1 mL samples were aliquoted from the pooled plasma of each patient. A sodium nitrate master mix (6.4 mol/L) was produced by dissolving 5.44 g of sodium nitrate into a final volume of 10 mL of deionised water. The master mix was sequentially diluted 1:2 five times, and 10 μL of each dilution (except the original master mix) was added to a different pooled plasma aliquot. The final concentrations of nitrate in each dilution series were 2, 4, 8, 16 and 32 mmol/L.

The median concentration of nitrate (8 mmol/L) in each dilution series was selected by calculating a theoretical plasma nitrate concentration from an ingested dose of 10 g of sodium nitrate with an oral bioavailability of 100%⁵ and a volume of distribution of 0.3 L/kg body weight.⁶ Samples were run in triplicate on each instrument, and the mean result for each concentration of nitrate was calculated from the total results of all three pooled patient samples ($n=9$). For each concentration of nitrate, the mean Alinity chloride result was then subtracted from the mean ABL800 chloride result. Linear regression analysis was used to model the relationship between nitrate concentration and ABL800 chloride interference. Analysis of variance (ANOVA) was used to assess the chloride concentration by Alinity between samples of differing nitrate concentrations.

There were no significant differences in chloride concentration between samples of different nitrate concentrations (ANOVA, $p=0.990$) by the Alinity method. The chloride results exhibited a positive, dose-dependent relationship with nitrate concentration (Fig. 1) with the ABL800 method. The 32 mmol/L nitrate samples all produced chloride results greater than the upper limit of detection (350 mmol/L) and were thus omitted from further analysis. There was an increased variation in chloride readings between samples, with the highest included nitrate concentration (16 mmol/L), coefficient of variation (CV) of 14%, compared to CVs under 6% for lower concentrations of nitrate. For this reason, linear

Fig. 1 Measured chloride by Alinity (Abbott) and ABL800 (Radiometer) methods as a function of sodium nitrate concentration in samples spiked with sodium nitrate. Each data point represents the average of 9 readings (triplicate measurements of 3 different patients' pooled plasma samples). Error bars represent standard deviation.

regression included only nitrate concentrations up to 8 mmol/L. The derived equation '[nitrate] = 0.13ΔCl' (Fig. 2) has a coefficient of determination = 0.99.

The data here reiterate the nitrate interference phenomenon associated with chloride measurement on the Radiometer ABL800, and the lack of such interference on Abbott's current model of routine analyser, Alinity. The explanation for discordant chloride measurements between manufacturers is based on differences in ISE compositions and electrochemical principles. The Alinity analyser utilises a redox system, whereas the ABL800 uses a semi-permeable membrane to selectively measure chloride. Measurement on both systems is necessary to quantify the magnitude of interference by nitrate.

This study provides a simple calculation to estimate the approximate blood nitrate concentrations from the discrepancy in chloride measurements between a silver nitrate redox system method and an ion-selective polymer membrane to measure chloride. This could prove valuable in the acute care setting, especially as presentations of nitrate and nitrite toxicity are expected to increase and clinical laboratories do not routinely measure nitrate quantitatively. Further experiments are required to assess the accuracy of this nitrate estimation compared to directly measured nitrate. In particular, the relationship between nitrate concentration and chloride interference should be validated across ISEs of different lifespans, as this is a factor known to influence the magnitude of nitrate interference.¹ It will also be important to quantify the extent to which nitrite species interfere with chloride measurement, as it is thought to be the compound that causes methaemoglobinaemia in cases of nitrate ingestion.

Fig. 2 Linear regression was used to predict plasma nitrate from a difference in chloride concentration between instruments. The relationship $[\text{NO}_3^-] = 0.13 \Delta [\text{Cl}^-]$ was calculated to have a $r^2 = 0.99$. Error bars represent standard deviation. Samples with nitrate concentrations of 16 and 32 mmol/L were omitted because of a disproportionately high CV and measurements above the upper limit of detection, respectively.

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