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Elevated ammonia concentrations: Potential for pre-analytical and analytical contributing factors

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ABSTRACT

Background: No study has explored the separate contributions of pre-analytical and analytical factors to hyperammonemia.

Methods: Laboratory information systems were queried for tests of ammonia concentrations over a 12 month period. Pre-analytic (collection to laboratory receipt) and analytic (laboratory receipt to result) elapsed times were determined.

Results: Under routine conditions for 3626 tests, normal and elevated results were similarly distributed if the time from venipuncture to result was <120 min. Delays, during analysis performance and in transportation to the laboratory, potentially contributed to hyperammonemia in a small number of samples ($n = 96$, 2.7%). Similar results were obtained from a second hospital with a separate laboratory.

Conclusions: Delays, in either transportation to the laboratory after collection or before completion of analysis, have the potential to elevate ammonia concentrations and may cause pseudo-hyperammonemia. Unexpectedly elevated ammonia concentrations need to be evaluated for errors in sampling handling.

Introduction

Measurement of circulating ammonia level is performed as part of the investigation and management of patients with altered mentation and hepatic dysfunction [1], patients receiving chemotherapeutic agents [2] and certain medications such as sodium valproate [3], and in patients suspected of inherited metabolic disorders [4,5]. Falsely elevated plasma ammonia concentrations can be due to pre-analytical events such as delayed sample processing [6] and to environmental contamination following cigarette smoking [7] and use of ammonia containing cleaning reagents. Considering the importance of this biochemical marker and the consequences of false positive results on a patient's investigation and treatment, care should be taken to minimize artifacts caused by pre-analytical factors. Immediate intervention may be required and therefore accurate determination is important.

Ammonia is an end-product of deamination of amino acids. *In vivo*, it is normally produced, predominantly in the gastrointestinal tract [8], myocytes [9], lymphocytes [10], and the kidney [11]. Ammonia is neurotoxic [12] if it accumulates but normally it is metabolized to urea by hepatocytes and to glutamine by myocytes and hepatocytes. Urea is excreted by the kidney but can also diffuse into all tissues and fluids. In the gastrointestinal tract, bacterial ureases produce ammonia from the urea. Significant formation of ammonia occurs following hydrolysis of glutamine by glutaminase containing cells such as gastrointestinal epithelial cells and lymphocytes.

The literature [5,13,14] and manufacturers of ammonia assays recommend: a.) use ethylenediaminetetraacetic acid (EDTA) as a preservative, since heparin interferes in the ammonia assay; b.) collect the sample and transport on ice; c.) separate the plasma from cells within 15 min of sample collection because blood cells metabolize glutamine into ammonia, and the rate of *in vitro* ammonia production correlates with platelet and white blood cell counts [15]; and d.) perform the assay within 20–30 min of venipuncture. Placing the sample on ice and immediate removal of plasma from cells reduce the reaction rate and remove a potential source of ammonia synthesis respectively.

This study investigates the effect of elapsed time on results of 3625 clinical assays to establish a time threshold beyond which a false elevation in ammonia may occur.

Materials and methods

A UT Southwestern institutional review board approved retrospective electronic review was performed of all ammonia results obtained from orders placed in the laboratory information system (Cerner Millennium) at Parkland Memorial Hospital, Parkland Health and Hospital System (PHHS) in the 2012 calendar year.

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Specimen collection and transport

Blood samples for ammonia measurement were collected in EDTA-plasma tubes and transported on ice-slurry. Mean transportation time from blood collection to receipt into the laboratory was 12 min (median = 9 min) and mean time from receipt to results reporting was 53 min (median = 44 min). This time includes refrigerated centrifugation (4 °C) for 10 min at 2600 rpm.

Measurement of ammonia

Ammonia was measured using a glutamate dehydrogenase enzymatic method using COBAS® (Roche Diagnostics, Indianapolis). Sample collection, transportation and analysis were according to the manufacturer's instructions. In this method 2-oxogluturate is converted to glutamate and $NADP^+$ produced is proportional to the ammonia concentration.

Reference intervals were adopted from published manufacturer ranges and verified using normal volunteers as transferable (13–40 μmol/L). The analytical measuring range of the assay is 10–700 μmol/L and the clinically reportable range is 10–1400 μmol/L. Precision is 12.4% and 6.7% at mean ammonia levels of 43 and 104 μmol/L respectively. Interference due to hemolysis, icterus and lipemia is automatically detected by the instrument and samples beyond acceptable limits are rejected.

Data collection and analysis

At PHHS, Cerner Millennium® laboratory information system was queried for all orders for ammonia placed in the 2012 calendar year. Results of each ammonia test and its associated times for ordering, blood collection, laboratory receipt and resulting were obtained. Quality assurance tests and tests performed at a collaborating institution because of lipemia were deleted from the analysis set. In addition, rejected specimens (e.g. hemolysis) were not resulted and therefore ineligible for analysis. We calculated the elapsed time between each of the standard periods after blood collection (collection to laboratory receipt to result) and compared the relative percentage of normal to elevated results for the various elapsed time parameters. Identical procedures were used for UT Southwestern with Sunquest Laboratory™ information system.

Statistical analysis

Categorical variables were compared with Pearson chi-square tests. Linear relationships between ammonia levels and time were determined by linear regression. Statistical analysis was performed using Stata 12.1 (College Station, TX, USA).

Results

For the 2012 calendar year, we retrieved 3747 records after querying the laboratory information system (see Fig. 1 for overall schema). Of these, 62 were obtained for quality assurance and patient safety while 51 samples were lipemic and transported to a collaborating partner for analysis. Unsatisfactory specimens ($n = 9$, e.g. hemolyzed) were not resulted. The remaining 3625 tests were obtained on 1835 individuals with the number of tests per subject averaging 2 and ranging from 1 to 30. As shown in Fig. 1, when we analyzed the time that elapsed between collection and result, significantly more samples with elevated ammonia concentrations were in the longer time period category, >120 min ($p < 0.001$).

Analyses of results for any given elapsed time are shown in Table 1. The total time from collection to reporting the result was divided into 30 minute increments. When the total time was <30 min, 68% of the results were abnormal, with a trend toward significance when compared to the majority of samples with a total time between 30 and 59 min ($p = 0.058$). Further analysis indicated that this was due to two separate factors, the priority of the order (routine versus stat) and whether the subject was in the Emergency Service Department, close to the laboratory, or not (data not shown).

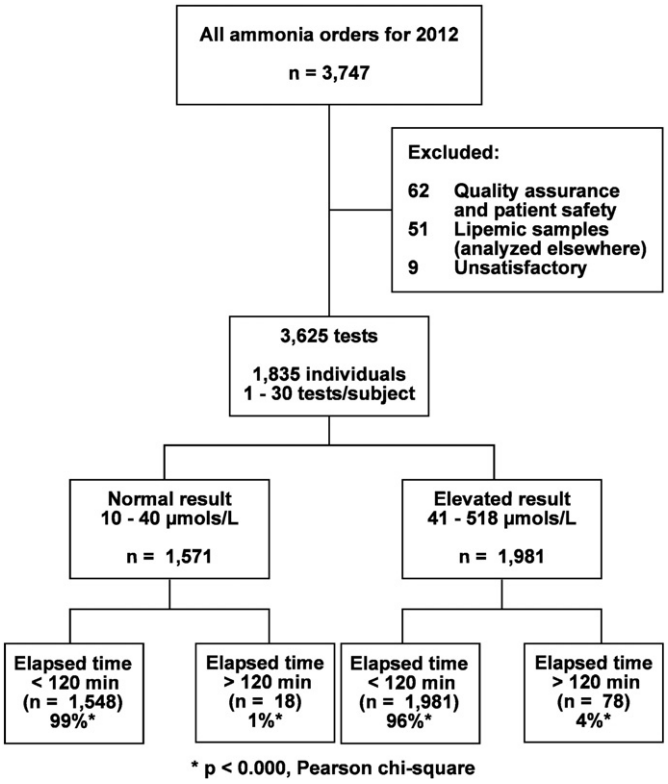

Fig. 1. Overall flow diagram for analyses. Laboratory information system records for all ammonia levels resulted in the 2012 calendar year were retrieved. After exclusion of ineligible records, the total elapsed time from collection to result was calculated. Normal ammonia levels (≤40 μmol/L) and elevated levels were divided into those with a total elapsed time <120 min and >120 min. The percentage of normal versus elevated results was significantly different between the two groups ($p < 0.001$).

For the 30 minute increments between >30 min and < 120 min, there were no significant differences ($p > 0.5$), each had 56%–58% abnormal results. In contrast, the increment from 120 to 149 min was again significantly different when compared with immediately preceding increment with 74% abnormal results ($p = 0.028$). These data established the 120 minute cut-off for reliability of results (Table 2). Similar results were obtained in both laboratories (Table 2 and data not shown).

The elapsed time includes 2 separate components, pre-analytic and analytic. The pre-analytic period starts with collection and ends with

Table 1
Elapsed time effects on results (PHHS).

Total time (min)	Normal result ≤40 μmol/L	Elevated result >40 μmol/L	All results (% total)
<30	20 (32%)*	42 (68%)*	62 (2%)
30–59	1348 (45%)*	1671 (55%)*	3019 (84%)
60–89	109 (43%)	146 (57%)	255 (7%)
90–119	49 (41%)†	71 (59%)†	120 (3%)
120–149	21 (26%)†	59 (74%)†	80 (2%)
150–179	6 (17%)	29 (83%)	35 (1%)
>180	6 (19%)	25 (81%)	31 (0.9%)
All times	1566 (43%)	2059 (57%)	3625

Total elapsed time was calculated for all eligible samples and divided into 30 minute time intervals up to 180 min. The number of normal and elevated ammonia levels and their relative percentage were calculated for each time interval and differences compared for significance.

* $p = 0.058$, Pearson chi square comparison of <30 min versus 30–59 min.

† $p = 0.034$, Pearson chi square comparison of 90–119 min versus 120–149 min.