Determination of ammonia concentrations in cirrhosis patients—still confusing after all these years?

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ABSTRACT

By the end of the nineteenth century, ammonia had been identified as the main factor responsible for hepatic encephalopathy syndrome. Ammonia is one of the principal products of hepatic metabolism and high concentrations are toxic to the body. Under physiological conditions, the main way by which the body restricts the blood concentration of ammonia to a nontoxic level is by converting it to urea in the liver via the Krebs cycle. The synthesis of glutamine represents an alternative pathway for ammonia detoxification in cirrhotic patients. Although high concentrations of ammonia have been strongly associated with brain edema, estimates of the strength of the correlation between serum ammonia levels and the severity of hepatic encephalopathy vary. The accuracy of ammonia assays depends on the site of specimen collection, treatment of the specimen and the analytical method used. New methods involving measurement of the partial pressure of ammonia and new noninvasive techniques involving quantification of ammonium in the breath have been described. The purpose of this review is to identify factors that affect serum ammonia levels, from its origin and metabolism to its analysis and interpretation of results in the laboratory. In conclusion, variations in estimates of serum ammonia level and the severity of hepatic encephalopathy arise because of individual differences in ammonia metabolism and differences in the accuracy of analytical methods.

AMMONIA: ORIGIN, METABOLISM AND ELIMINATION

Ammonia is one of the main products of hepatic metabolism and is toxic in high concentrations. Eighty-five percent of the intestinal production of ammonia is generated from deamination of glutamine (Gln) by phosphate-activated glutaminase (PAG) in the small intestine. PAG catalyzes the hydrolysis of Gln to produce glutamate and ammonia. As this enzyme is present in the liver, kidney, brain and enteric villi, the intestine is not the only site of ammonia production. Under physiological conditions, the kidney generates ammonia, predominantly via PAG. Thirty percent of the ammonia generated in the kidney by PAG is excreted in the urine, and the remainder enters the systemic circulation through the renal vein.

When the liver is fully functional, the concentration of ammonia is restricted to a nontoxic level by its conversion to urea via the Krebs cycle. Production of ammonia by PAG activity and its removal by urea synthesis take place in the perportal hepatocytes. Ammonia that escapes periportal removal by the urea cycle is removed by perivenous hepatocytes via glutamine synthetase. Gln synthesis represents an alternative means of ammonia detoxification in cirrhotic patients. Gln acts as a nontoxic ammonium transporter in the circulation.

In patients with cirrhosis, the reduction in hepatocellular function and the generation of portosystemic shunts contribute to increased serum levels of this neurotoxin. In this context, high blood ammonia levels may be an indirect measure of the presence of collateral blood vessels and esophageal varices, and could help to identify cirrhotic patients who require closer endoscopic follow-up. When hepatic activity is insufficient to maintain ammonia homeostasis, other organs are adapted to respond to this situation. In patients with cirrhosis, the ammonia that cannot be converted to a nontoxic compound in the liver is released into the systemic circulation and is taken up by muscle. Muscle constitutes more than one-third of body weight and therefore is one of the main sources of Gln because of the activity of glutamine synthetase and protein catabolism. As the Gln produced by muscle may be released and subsequently used as a substrate for the generation of ammonia in the intestine and kidney, its net effect on systemic ammonium mobilization is limited.

The kidneys make the main contribution to balancing serum ammonia levels. In hyperammonemia, the relationship between generation and renal clearance of ammonia is altered such that the release of ammonia into the circulation by the renal vein is decreased and its urinary excretion is increased, as occurs in response to metabolic acidosis, hypokalemia and extracellular volume expansion. The increase in the renal elimination of ammonia in the urine stimulates the generation of bicarbonate, which in turn can be used in the hepatic synthesis of urea. Jalan, et al. showed that saline infusion decreases plasma ammonia level from 93 µmol/L to 56 µmol/L (p < 0.05) and increases urinary excretion of ammonia from 0.9 mmol/h to 1.4 mmol/h (p < 0.05). It follows that, in patients with acute HE, a favorable clinical outcome will be obtained in response to hydration and that the kidney should be the target organ for new therapeutic modalities.

Ammonia has been strongly related to brain edema secondary to liver failure. A histological study of patients who died of HE revealed evidence of edema of astrocytes, neural cells considered most sensitive to hepatic failure. Glutamate is the major excitatory neurotransmitter in the central nervous system. Reuptake of glutamate by the astrocyte prevents its accumulation in the synaptic space and protects neurons from excessive activation. Within the astrocyte, glutamate is a precursor of Gln and glutathione. Gln synthesis is the main mechanism responsible for ammonia detoxification in the central nervous system. Glutamine synthetase activity in the brain is confined to astrocytes. Gln is released into the extracellular space and is taken up by neurons to produce glutamate and ammonium, a reaction mediated by PAG. Both HE and the hyperammonemic states are associated with elevated levels of Gln in the brain and spinal fluid.

As Gln has an osmotic effect, high levels could cause cellular edema. In response to accumulation of Gln and the resultant hypotonicity, glial cells release osmolytes such as myoinositol and taurine into the extracellular space. This partially restores intracellular osmolarity but does not prevent subsequent cell swelling.

In cirrhosis patients, dilutional hyponatremia is a typical response to inappropriate antidiuretic hormone secretion. Hyponatremia has been identified as an independent risk factor for HE. During hyponatremia and edema, cerebral osmolytes are released into the extracellular space. A decrease in the intracellular level of osmolytes during hyponatremia may limit the ability of astrocytes to adapt to the increase in intracellular osmolality caused by Gln in hyperammonemic states.
As small changes in the extracellular environment of the brain can affect neurotransmission, the BBB, which is composed of endothelial cells connected by tight junctions, limits the entrance of substances that are potentially harmful for the central nervous system. In the past, it was believed that ammonia was unable pass through the BBB because it is impermeable to ions, and ammonia is mainly present in the ionized form, NH4+, in the systemic circulation. However, Lockwood, et al. showed that 13N-labeled ammonia was able to cross the BBB. Ammonia that passes through the BBB is used by the brain and its utilization rate increases in parallel with arterial ammonia concentration. Cerebral metabolic rate is elevated in patients with portosystemic encephalopathy and is accompanied by an increase in permeability per unit surface area, a measure of BBB permeability.

Astrocytes are a component of the BBB and regulate the brain’s blood flow via an arachidonic acid-dependent pathway. As presence of markers of the systemic inflammatory response is a predictor of deterioration in the degree of HE, it is assumed that inflammation exacerbates the neurological changes induced by hyperammonemia. This may be the result of disruption of the BBB during acute infectious episodes. Although treatment with nonsteroidal anti-inflammatory drugs has shown improvement in cognitive function in animal models of portosystemic encephalopathy, their use in humans has been limited because of concerns about their effect on kidney function.

The magnitude of the clinical manifestations of a sudden increase in serum ammonia level differs from one patient to another. These differences may result from genetic alterations in the key enzymes of Gln metabolism. Using experimental animal models, several polymorphisms in the promoter of the PAG gene have been identified which may explain variation in the activity of this enzyme in humans. The intestinal activity of PAG is elevated in patients with cirrhosis and is indicative of minimal HE. Studies on polymorphisms in the PAG gene have revealed that the TACC haplotype is associated with improved liver function, reduced risk of HE and low intestinal ammonia production.

The oral Gln challenge is a method that increases blood ammonia concentration and is an indirect measure of intestinal PAG activity. In patients with minimal HE, a positive oral Gln challenge result is associated with a 60% risk of an episode of acute HE within one year. The oral Gln load affects the results of neuropsychological and neurological tests, especially in patients with EEG abnormalities or minimal HE, indicating that cirrhotic patients become sensitized to ammonia.

Hypersensitivity to this neurotoxin could be mediated by cell membrane water channels called aquaporins. These channels regulate the flow of water to and from the brain and are intimately involved with the development of cerebral edema in patients with cirrhosis. Hyperammonemia upregulates aquaporin 4 expression. Increased aquaporin expression results in osmotic gradients across cell membranes. The precise regulatory mechanisms of these proteins have not been clarified, but they are probably responsible for astrocyte edema and increased BBB permeability in liver failure.

**DETERMINATION OF AMMONIA**

The ammonia concentrations of cirrhosis patients have been measured since the beginning of last century, and it has been observed that ammonia concentrations are affected by factors other than the analytical method used. Ammonia levels are four to eight times higher in neonates than in adults, two to three times higher in children under the age of three years than in adults, and are commensurate with those of adults during adolescence. Exercise increases the level of ammonia by up to three times, and the increase is highest among men. Ammonia level increases by 10 µmol/L after smoking a cigarette. The absorption of glycine during transurethral prostatectomy can cause a transient elevation in ammonia level and induce metabolic encephalopathy. The administration of valproic acid is associated with hyperammonemia, especially in patients with carnitine deficiency. Idiopathic hyperammonemia syndrome occurs when abnormalities in liver function are absent, and has been associated with the use of chemotherapy after bone marrow transplantation.

Ammonia concentration is affected by the site from which the specimen is collected, the way in which the specimen is processed and the analytical method used. If a blood sample is centrifuged immediately and the plasma is refrigerated at 4°C, ammonia concentration will remain relatively stable for up to 60 min, and the increase in ammonia concentration will be limited to 2 µmol/L (5%). In patients with liver disease, centrifugation of the sample within 15 min of collection is ideal. The sample should be placed on ice immediately after collection because erythrocyte and platelet metabolism persist in vitro and may increase ammonia...
concentration by 20% within 1 h and by up to 100% within 2 h if the sample is held at room temperature. There is a positive relationship between the rate of ammonia formation in vitro and the subject’s level of alanine aminotransferase (ALT). 41

In 1963, Stahl reported that arterial blood was most representative of whole body ammonia levels because the concentration of ammonia in venous blood may be affected by peripheral metabolism and the uptake of ammonia by muscle and brain tissue. 9 In biological fluids, ammonia exists in two forms: as ionized ammonium, \( \text{NH}_4^+ \), and in its gaseous unionized form, \( \text{NH}_3 \). At a physiological pH of 7.4, 98% of plasma ammonia is in the ionized form. 42 The level of \( \text{NH}_3 \), which freely crosses the BBB, is a function of the partial pressure of ammonia in the blood. Kramer, et al. measured the arterial partial pressures of \( \text{NH}_3 \) gas and total arterial ammonia concentrations in patients with acute HE (grades I–IV). 43 They found that the clinical grade of encephalopathy was better correlated with the partial pressure of \( \text{NH}_3 \) than with total arterial ammonia concentrations (\( r = 0.79 \) vs. \( r = 0.69 \), respectively; \( p = 0.01 \)). Subsequently, Ong, et al. compared arterial and venous ammonia concentrations with the partial pressure of \( \text{NH}_3 \) in arterial and venous blood. 44 Results obtained using these four approaches were all significantly correlated with the West Haven scale (\( p \leq 0.001 \)). The correlation coefficients were arterial total blood ammonia, \( r = 0.61 \); venous blood ammonia concentration, \( r = 0.56 \); arterial partial pressure of \( \text{NH}_3 \), \( r = 0.55 \); and venous partial pressure of \( \text{NH}_3 \), \( r = 0.52 \). It is important to note that the population analyzed by Kramer was composed entirely of patients with acute HE, and that analyzed by Ong, et al. was composed of cirrhotic patients with or without clinically manifest HE. This difference could have caused the difference in their estimates of the correlation coefficient between grade of encephalopathy and arterial partial pressure of \( \text{NH}_3 \) (\( r = 0.79 \) vs. \( r = 0.55 \), respectively).

Several efforts have been made to develop more sensitive and less invasive specimen collection techniques. Dubois, et al. tested the efficacy of measuring ammonia concentrations in expired breath using an optic fiber sensor on 17 cirrhosis patients, and reported a negative correlation between breath test results and the time required to complete the number connection test (\( r = -0.55, p = 0.03 \)). 45

An attractive alternative method for measuring ammonia concentration involves a pocket device that enables bedside specimen processing. The pocket analyzer is a portable device for determining ammonia concentration in whole blood. It requires a small blood sample (20 mL) and produces results within 3 min. The immediate analysis of the sample decreases error arising from spontaneous generation of ammonia by in vitro metabolism of erythrocytes. This method is based on microdiffusion and colorimetry. The lower and upper detection limits of the unit are 7 \( \mu \text{mol/L} \) and 286 \( \mu \text{mol/L} \), respectively. Goggis, et al. compared the accuracy of the pocket analyzer with that of an enzymatic analytical method, 46 and found that results obtained using the pocket device were positively correlated with the reference method (intraclass correlation coefficient, 0.800; 95% confidence interval, 0.655-0.888). The pocket analyzer has a proportional negative bias, i.e., it underestimates ammonia level to a greater extent at high concentrations than at low concentrations.

The most widely used analytical method for detecting ammonium is an enzymatic method. This analysis is based on the reaction of glutamate dehydrogenase, which catalyzes the condensation of \( \text{NH}_4 \) and 2-oxoglutarate to form glutamate in the presence of NADH or NADPH. The concentration of ammonia is proportional to the oxidation of NADPH to NADP, the concentration of which is measured as the change in absorbance at 340 nm using a spectrophotometer. High levels of ALT in serum may interfere with this measurement. Pyruvate is a by-product of ALT activity and is reduced by NADH to lactate in the presence of lactate dehydrogenase (present in the ammonia reagent), which increases the amount of NAD in the solution, resulting in an overestimation of ammonia concentration. 47

The dry chemistry method uses a reactive multilayer incorporated into a polyester base. Ammonia reacts with a bromophenol blue indicator to produce a colored compound, which is measured by reflection spectrophotometry at 600 nm. This method is safer, simpler and less expensive than the enzymatic method and produces results within 5 min. 48

Herrera, et al. compared the enzymatic and dry chemistry methods in patients with clinical conditions such as acute liver failure or multiorgan failure, which are associated with high or moderate elevation of ALT level. 47 They added solutions with progressively elevated concentrations of ALT to plasma samples to assess the effect of ALT on ammonia concentration. With the enzymatic method, the addition of ALT elevated ammonium concentration by 96 \( \mu \text{mol/L} \) to 391 \( \mu \text{mol/L} \). The activities of other dehydrogenases present in the sample could be responsible for these differences. Two-step enzymatic methods are available that enable corrections to be
made to allow for nonspecific oxidation of NADPH to NAD, which generates results similar to those obtained using the dry chemistry method.49

CONCLUSIONS

The availability of more sensitive and specific analytical methods has increased the diagnostic accuracy of blood ammonia concentrations. We suggest the use of the dry chemistry or the two-step enzymatic method to measure ammonia. Although improvements in analytical methods have resulted in stronger correlations between hyperammonemia and the severity of HE, there is still considerable variation between these estimates, possibly because of interobserver variability in assessing the severity of HE. Neuropsychological tests are influenced by the patient’s age and education level. The main difficulty is that there is no standard definition of HE in neuropsychological terms. Therefore, it must be recognized that the sensitivities of methods for clinical evaluation of HE are such that concordant results from more than one method are required before a diagnosis of HE can be made with confidence.

We recommend using blood ammonia concentrations:

- In conjunction with the results of clinical, neuropsychological and neurophysiological tests.
- To assess the effectiveness of drugs for research purposes.
- To monitor fluctuations that may occur in an individual patient, as there is no threshold of ammonia level for cognitive impairment.

Changes in a patient’s ammonia concentration may be a predictor of the onset of clinical manifestations. The disparity between serum ammonia levels and clinical severity probably resides in failure to take into account the influence of systemic ammonia metabolism, individual genetic variations in the expression of enzymes involved in Gln metabolism and cell membrane proteins associated with regulation of osmotic gradients in the brain, and the role of other unidentified neurotoxic substances.

ABBREVIATIONS

- EH: Hepatic encephalopathy.
- BBB: Blood-brain barrier.
- PAG: Phosphate-activated glutaminase.
- Gln: Glutamine.
- ALT: Alanine aminotransferase.

REFERENCES


