Measurement and interpretation of plasma ammonia

Introduction

Ammonia is a by-product of nitrogen metabolism originating from several biochemical pathways that take place in the intestines, kidney, muscle and brain. This article reviews the clinical utility of plasma ammonia measurement, and its interpretation. It provides guidance on how to minimize errors in its measurement and on the management of hyperammonaemia in the context of liver disease.

Ammonia metabolism

Ammonia is toxic when levels accumulate and it is removed from the body by being converted to urea in the liver and then renally cleared. Most ammonia in the body is produced in the intestines through deamination of nitrogenous compounds and hydrolysis of urea by bacteria. It then crosses the intestinal epithelium to enter the portal circulation. Ammonia is also produced by cellular degradation of amino acids as part of protein catabolism and most cells synthesize glutamine to carry ammonia to the liver for removal. Skeletal muscle also produces alanine for this purpose.

The kidneys produce ammonia via the breakdown of glutamine within the proximal tubule. In normal physiological states, 70% of this ammonia enters the renal vein, with the remaining 30% excreted in urine (Dejong et al, 1993). In muscle, ammonia production increases during exercise. The amount is dependent on duration and intensity, and arises through adenosine monophosphate deamination and branched chain amino acid metabolism (Wilkinson et al, 2010). Blood urea clearance by muscle glutamine synthetase is of particular importance in liver failure and although its activity is low, the net effect is significant owing to the total body muscle mass (Chatauret and Butterworth, 2004). Finally, ammonia production also takes place within neurons; here its removal is catalysed by glutamine synthetase in astrocytes (Chatauret and Butterworth, 2004).

Ammonia in the portal circulation is taken up by periportal hepatocytes where it is incorporated into the urea cycle and perivenous hepatocytes use it to synthesize glutamine (*Figure 1*). Circulating glutamine and alanine are taken up and deaminated via glutaminase and the glucose-alanine cycle respectively to generate ammonia which feeds into the urea cycle. The healthy liver plays a major role in nitrogen homeostasis, and keeps plasma ammonia levels below 100 µmol/litre (Felipo and Butterworth, 2002).

Hyperammonaemia

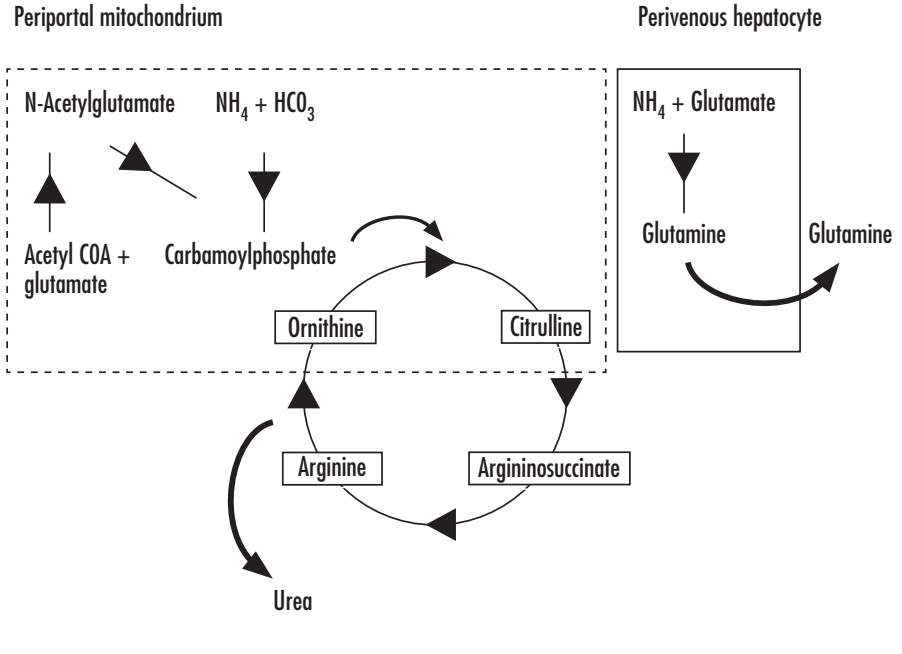
Elevated ammonia levels may arise from either increased production or reduced clearance. Interpretation of hyperammonaemia depends on its clinical manifestation. Given that the blood–brain barrier is permeable to ammonia, its accumulation can lead changes in neurotransmission and astrocyte swelling and consequently altered mental status.

Role of ammonia in liver disease and hepatic encephalopathy

Owing to the central role of the liver in ammonia metabolism, patients with chronic liver disease may become hyperammonaemic. Hyperammonaemia is a component of hepatic encephalopathy, a disabling neuropsychiatric disorder that is characterized by personality changes, impaired intellect and altered levels of consciousness which can lead to coma and death (Ferenci et al, 2002; Cash et al, 2010). The degree of mental status disturbance is classified using the West-Haven criteria (*Table 1*).

Interpretation of serum ammonia measurements is difficult as there is poor correlation between plasma concentration and clinical manifestations: many patients with chronic liver disease have elevated blood ammonia levels in the absence of clinical evidence of encephalopathy, there is considerable overlap between ammonia concentrations seen in the different grades of hepatic encephalopathy and some

Figure 1. Hepatic uptake of ammonia, ureagenesis and glutamine synthesis.



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encephalopathic patients have ammonia levels within the normal range. Consequently a high blood ammonia level neither establishes the diagnosis of hepatic encephalopathy, nor is a normal level adequate for exclusion (Ong et al, 2003) and ultimately the diagnosis remains one of clinical judgement.

As such, ammonia measurements rarely alter patient management but may contribute to the overall clinical assessment. Indeed, there are risks of falsely attributing altered mental status in a patient with chronic liver disease to encephalopathy on the basis of hyperammonaemia, without first considering or excluding other common causes in this patient group such as subdural haematoma, spontaneous bacterial peritonitis, sepsis, hypoglycaemia, drug toxicity and Wernicke's encephalopathy (Elgouhari and O'Shea, 2009). Plasma ammonia levels are helpful in cases of acute liver failure, where they provide prognostic information thought to correlate with astrocyte swelling, raised intracranial pressure and risk of cerebral herniation.

Other causes of hyperammonaemia

Encephalopathy and hyperammonaemia without liver disease can be attributed to increased ammonia production or decreased excretion. The underlying cause may be reversible and thus specific treatment to reduce ammonia levels will be indicated.

Increased production

Hyperammonaemia can arise as a metabolic complication of ureterosigmoidostomy as large quantities of urinary nitrogenous compounds become degraded by bowel bacteria (Hawkes et al, 2001). Total parenteral nutrition may also lead to hyperammonaemia, since the body is unable to regulate the normal digestion and absorption of protein which takes place with dietary intake (LaBuzetta et al, 2010). Generalized convulsions have also been noted to cause transient hyperammonaemia, which is associated with postictal confusion (Liu et al, 2011). Thus even in the context of normal liver function, if ammonia production exceeds the capacity of metabolic pathways, it may diffuse directly into the inferior vena cava and subsequently the brain.

Decreased clearance

Decreased ammonia excretion accounts for the bulk of non-hepatic hyperammonaemic states.

Portosystemic shunts

In the context of liver disease, shunt formation occurs as a consequence of portal hypertension. Causes of portosystemic shunts in the absence of cirrhosis occur in congenital abnormalities of intrahepatic vascular system, vascular anastomoses after abdominal surgery, liver biopsy and trauma (Watanabe, 2000) or, commonly, iatrogenically following the creation of a transjugular intrahepatic portosystemic shunt.

Drug induced

Several medications can disrupt the urea cycle. Hyperammonaemia may occur in patients with haematological malignancies during cytotoxic chemotherapy and bone marrow transplantation, and in patients with tumours being treated with 5-fluorouracil (Nott et al, 2007). Sodium valproate decreases the availability of N-acetylglutamate and consequently the activity of carbamoyl phosphate synthetase I, which is required for the first step of the urea cycle (LaBuzetta et al, 2010). It also increases renal excretion of carnitine resulting in compensatory amino acid oxidation and increased production of nitrogenous compounds. The prevalence of asymptomatic hyperammonaemia is high (Murphy and Marquardt, 1982), although it can rarely cause encephalopathy. In such cases, L-carnitine supplementation is helpful.

Inborn errors of metabolism

Synthesis of urea in the liver is the major route of ammonia excretion. Inborn errors of metabolism causing hyperammonaemia can be caused by urea cycle defects, organic acidaemias, fatty acid oxidation and transporter defects. In neonates, inborn errors of metabolism may present with lethargy, irritability, temperatures and feeding problems; in older children inborn errors of metabolism may present with failure to thrive, poor global development, behavioural disturbances or even convulsions (Broomfield and Grunewald, 2011). Of the urea cycle disorders, the X-linked ornithine transcarbamylase deficiency which usually presents in childhood is the most common (LaBuzetta et al, 2010). Ammonia levels in these cases should not be missed since this deficiency is reversible and measuring urea cycle intermediates can facilitate the final diagnosis.

How to measure ammonia

The challenge in ammonia measurement is in reducing false positives which occur because of patient confounding factors and sample handling. High protein intake, smoking, prolonged tourniquet application and fist clenching all increase ammonia levels (Elgouhari and O'Shea, 2009). Unless taken as an emergency sample, patients should be in a fasted state and advised to stay as relaxed as possible to minimize muscle activity (Lowenstein, 1972). Venous blood is as accurate as arterial for ammonia measurement (Ong et al, 2003).

As per recommendations from the UK National Metabolic Biochemistry Network, the blood sample should be drawn into a chilled vacuum tube containing either lithium heparin or EDTA, placed onto ice and transported immediately to the laboratory for analysis. Plasma should be separated within 15 minutes of collection (Wright, 2010). These recommendations arise from the fact that in standing blood, ammonia concentrations can increase through its generation and release from erythrocytes and deamination of amino acids (McCullough, 1968). Delays in sample processing can lead to false positives (Maranda et al, 2007).

Table 1. West-Haven criteria

Grade 1: Trivial lack of awareness; euphoria or anxiety; shortened attention span
Grade 2: Lethargy or apathy; minimal disorientation; subtle personality change; inappropriate behaviour
Grade 3: Somnolence to semi stupor but responsive to verbal stimuli; confusion; gross disorientation
Grade 4: Coma

From Ferenci et al (2002)

Most laboratories in the UK measure ammonia using the reaction of glutamate dehydrogenase on ammonia, 2-oxoglutarate, reduced nicotinamide adenine dinucleotide phosphate (NADPH). The reduction in NADPH is proportional to the plasma ammonia concentration; NADPH can be measured by absorbance spectroscopy. Ammonia can also be measured using an indirect method where free ammonia is liberated through alkalization, it travels through a membrane and reacts with an indicator reagent. The intensity of the colour formed through this reaction is measured by reflectance spectroscopy to provide ammonia concentrations (Wright, 2010; Broomfield and Grunewald, 2011).

Management of hyperammonaemia in liver disease

Treatment of encephalopathy is aimed at reducing ammonia production and increasing its removal. There should be clinical evidence of improved mental status within 24–48 hours; should delirium persist longer than this, alternative explanations should be sought (Prakash and Mullen, 2010).

Reducing ammonia production

Non-absorbable disaccharides

The laxative effect removes ammonia before it is absorbed into the systemic circulation, it reduces the colonic pH to suppress urease-containing bacteria and reduces the amount of glutamine metabolised to ammonia within hepatocytes. Patients should aim for 2–3 soft stools per day. Although compliance is a problem, this approach improves cognitive function and quality of life (Prasad et al, 2007). Although a Cochrane analysis concluded that lactulose appears inferior to antibiotics in improving hepatic encephalopathy, it remains the first-line treatment at present (Als-Nielsen et al, 2005).

Antibiotics and probiotics

Antibiotics are aimed at the urease-containing bacteria; they work by causing cell toxicity, inhibiting protein synthesis or cell wall synthesis. The choice of antibiotics is limited because of systemic absorption and side effects; neomycin was used in the past, but it was unfavourable owing to nephrotoxicity and ototoxicity. Rifaximin is a newer approach, it is a poorly absorbed oral antibiotic that interferes with bacterial RNA polymerase and gene transcription. It has fewer adverse effects and consequently higher compliance rates than lactulose. It also reduces frequency and duration of hospital admissions (Leevy and Phillips, 2007; Bass et al, 2010) and improves quality of life (Sanyal et al, 2011). Trials into the efficacy of probiotics are inconclusive (McGee et al, 2011; Holte et al, 2012) – they reduce the effects of urease-containing bacteria through enhancing the growth of other bacteria.

Ammonia scavengers

Both sodium benzoate and sodium phenylbutyrate provide alternative pathways for ammonia removal (Frederick, 2011). They conjugate with glycine and glutamine forming hippuric acid and phenylacetylglutamine respectively. They are water-soluble compounds and are renally excreted. Although not often used, they provide alternative pathways for ammonia removal, and may be as safe and effective as lactulose (Sushma et al, 1992).

Increasing ammonia removal

Branched chain amino acids

These compounds lower the blood ammonia level through the production of glutamine. The use of lactulose in addition to rifaximin or branched chain amino acids provides better outcomes than monotherapy (Gluud et al, 2012).

L-ornithine-L-aspartate

These are both substrates in the urea cycle – ammonia levels are reduced through the stimulation of ureagenesis. L-ornithine-L-aspartate has been shown to reduce serum ammonia levels, improve electroencephalographic activity (Poo et al, 2006) and improve hepatic encephalopathy (Jiang et al, 2009).

Other

Therapeutic cooling

In acute liver failure, intracranial hypertension accounts for 20–25% of deaths. Within the intensive care setting, therapeutic hypothermia may slow whole body metabolism and thus reduce the production and cerebral uptake of ammonia (Stravitz and Larsen, 2009).

Dialysis

For patients with encephalopathy that has not responded with other therapies, one may consider the use of extracorporeal albumin dialysis with the molecular absorbent recirculating system. Although it has not been shown to affect liver transplant-free survival (Bañares et al, 2013), it may lead to earlier improvement of hepatic encephalopathy (Hassanein et al, 2007).

Liver transplantation

Transplantation is the ultimate treatment for persistent hepatic encephalopathy. Prognosis post-transplantation is worse in those with severe encephalopathy (Stewart et al, 2007).

KEY POINTS

- Ammonia is a by-product of nitrogen metabolism; the liver plays a major role in its homeostasis.
- Ammonia levels are of limited help when hepatic encephalopathy is suspected, as they are unlikely to alter management.
- In the context of liver disease, a normal ammonia level does not exclude hepatic encephalopathy, and a raised level should not abbreviate the search for other causes of impaired consciousness.
- Other causes of hyperammonaemia include: ureterosigmoidostomy, total parenteral nutrition, seizures, portosystemic shunts, drugs and inborn errors of metabolism.
- Management of hyperammonaemia in liver disease is based on reducing ammonia production or increasing its removal from the body.

Conclusions

Ammonia is a toxic by-product of nitrogen metabolism. Ammonia levels may be elevated through increased production or decreased clearance when the normal homeostatic pathways fail, and may manifest with altered mental status. In some cases, such as in liver disease, the measurement of ammonia seldom changes the subsequent management. However, when a reason for altered mental status is not clear its measurement may provide a foundation for further investigation of other possible underlying diagnoses.

When measuring ammonia, it is crucial to minimize errors in sampling technique and delays in processing. Management of hyperammonaemia is based on decreasing its production or expediting its clearance from the body. **BJHM**

Conflict of interest: none.

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TOP TIPS

- Ammonia levels are useful if there is altered mental status in a patient not known to have liver disease, or in the setting of acute liver failure.
- When taking blood samples for ammonia, the patient should be fasted and relaxed.
- Blood samples should be drawn into a chilled bottle and transported promptly to the laboratory on ice.