How to use serum ammonia

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Received 14 April 2011
Accepted 24 October 2011
Published Online First
18 November 2011

Abstract

Hyperammonaemia is a potentially extremely important indicator of impairment in intermediate metabolism. However, lack of experience in sample handling and confusion about what level is significant, can lead to its devaluation as a test. The aim of this article is to help the non-metabolic specialist to decide when it is appropriate to investigate for hyperammonaemia, to discuss potential investigatory pitfalls and to help in interpretation of results.

Introduction

When considering any key diagnostic metabolite in the investigation of suspected metabolic conditions, early and correct sampling are the main determinants of a successful diagnosis and can very often affect the outcome. Adequate performance of any investigation is essential for both reliable and interpretable results. There are few better examples than that of plasma ammonia, an investigation that has been noted to be poorly performed even in specialised centres.[1 2]

The aim of this study is to help the non-metabolic specialist to decide when it is appropriate to investigate for hyperammonaemia (something unfortunately often investigated only after substantial delay), to discuss potential pitfalls and to help to interpret results. In addition, the authors provide some information on the interpretation of basic biochemical tests in the context of hyperammonaemia (table 1). However, as has been stated in previous studies in this series, there is no substitution for close communication between the laboratory doing an investigation and the clinical team which is requesting it.

Biochemical/physiological background

Ammonia, a highly toxic intermediary metabolite, is formed during the catabolism of the nitrogen-containing amine groups of amino acids, as a normal part of biochemical homoeostasis. At physiological pH, ammonia is mainly found as ammonium (NH_4^+), a small molecule (of molecular weight 18D), which can diffuse freely across the blood–brain barrier. To prevent ammonia accumulating, it is immediately incorporated into glutamine and reconverted to ammonia in the liver.[3] Here it is rapidly converted into urea by a series of reactions known as the urea cycle (figure 1). It is typically a breakdown in the flux through this pathway that leads to hyperammonaemia, though general liver failure and the actions of urease-positive intestinal bacteria can also produce significant hyperammonaemia.[4 5]The importance of normal ammonia homoeostasis can be from the deleterious pathological cascade that results when ammonia accumulates, with the central nervous system, being especially affected. Here, increased levels of ammonia result in both acute and chronic effects, such as osmotic effects due to glutamine accumulation, alterations in cerebral energy use and neurotransmission, impairment of axonal and dendritic growth and induction of neuronal apoptosis.[3 6]

Inborn errors of metabolism (IEMs) that cause hyperammonaemia (table 2), without obvious liver failure, can be due to primary enzyme deficiencies of the urea cycle enzymes, due to defects in its substrate carriers, or because of secondary disruption of the urea cycle. Examples of secondary disruption are by the organic acidaemias such as propionic acidaemia or methylmalonic acidaemia, where accumulating propionyl-coenzyme A competitively inhibits the production of N-acetylglutamate (the cofactor of carbamylphosphate synthetase, CPS[7], though there may be other contributing factors).[8] The mechanism behind the fatty acid oxidation defects leading to hyperammonaemia is less clear but a possibility is that it is due to a generalised mitochondrial dysfunction resulting from a compensatory increase in ω-oxidation secondary to carnitine deficiency[9] or due to deficiencies in acetyl CoA.[10] Examples of transporter defects affecting the flux of urea cycle substrates into the cell cytoplasm or the mitochondria are lysinuric protein intolerance and citrin deficiency, respectively.

Table 1 Routine biochemical investigations and their meaning in the context of hyperammonaemia

Investigation	Meaning if hyperammonaemia present
Blood gas	A respiratory alkalosis is suggestive of a urea cycle disorder and results from initial over stimulation of central respiratory drive by ammonia which may be subtle.
	A metabolic acidosis should prompt the calculation of an anion gap but in the context of hyperammonaemia it is suggestive of severe cardiovascular compromise and/or an organic acidaemia.
Anion gap	$(Na^+)-(HCO_3^-+Cl^-)$=8–11. An increased anion gap is strongly suggestive of an organic acidaemia
Glucose	While hypoglycaemia is a non-specific marker of disease it does raise the possibility of a fatty acid oxidation disorder and should prompt testing of both creatine kinase and urinary ketones. The later being absent/ unexpectedly low in fatty acid oxidation disorders and in the HIHA.
Lactate	Typically a manifestation of poor peripheral perfusion or sampling from a squeezed capillary sample, but raises the possibility of organic acidaemias due to secondary TCA cycle inhibition or a mitochondrial cytopathy
Hypo/hypercalcaemia	A disturbance in calcium regulation is often found in organic acidaemias. This should also prompt an evaluation for pancreatitis, that is consider amylase and lipase, especially in older patients as this is a well-known complication of organic acidaemias.
Liver function test including clotting	If abnormal, suspect a fatty acid oxidation disorder and a creatine kinase should be taken. However liver failure is a feature of many disorders resulting in liver dysfunction.
Urinary ketones	The presence of ketones in the urine of a neonate is suggestive of an organic academia.[14]
	Their absence can suggest a fatty acid oxidation disorder or HIHA.

HIHA, Hyperinsulinism hyperammonaemia syndrome; TCA, tricarboxylic acid.

Technological background: how is ammonia measured?

The majority of laboratories in the UK use a method based on the reductive amination of 2-oxoglutarate by glutamate dehydrogenase (GLDH). This reaction, as can be seen below, requires reduced nicotinamide adenine dinucleotide phosphate (NADPH) with the reduction in NAPDH being proportional to the plasma ammonia concentration:

2-Oxoglutarate+NH_3+NADPH
↑↓GLDH
Glutamate+NADP

The importance of the NADPH is that the change in its concentration can be measured spectroscopically, as the decrease of absorbance at 340 nm (the wavelength of NADPH) reflects its oxidisation to NADP.[11]

In smaller laboratories where the demand is limited, reflectance meters are often used, employing dry slide chemistry strips for whole blood ammonia. In this method the intensity of colour formed by the reaction between blood ammonia and bromocresol green or similar indicator reagent is measured. The advantages of this technique are that it is quick, cheap and accurate.[12] However, it does have one very important disadvantage, which is a cut-off range of 286 μmol/l.

It is of the upmost importance that clinicians are aware of the techniques used in their laboratories, though the laboratory should also be feeding back that there is a possibility of the ammonia being substantially higher, if they are using reflectance meters.

Indications and limitations

In whom should hyperammonaemia be suspected?

Plasma ammonia can be used both as part of diagnostic investigations and for monitoring of efficacy of treatment. Diagnostically, while the key characteristic of hyperammonaemia is an unexplained acute or progressive encephalopathy,[13] its clinical presentation is also determined by the age at presentation and the degree of hyperammonaemia. Whereas the neonatal presentation is very non-specific, with neonates usually rapidly deteriorating, mimicking sepsis, the range of presentation in older children is more varied encompassing gastrointestinal symptoms, liver disease and psychiatric disturbances as well as classical encephalopathy. In both infants and older children the British Inherited Metabolic Disease Group (BIMDG) decreased consciousness level and the MetBionet investigation of developmental delay guidelines are useful resources in help guiding non-specialists to appropriate investigations.

Newborns

In the neonatal period, given the limited clinical repertoire of response in the newborn, the initial symptoms

Table 2 Causes of hyperammonaemia

Artefact	Haemolysis/specimen contamination/ delayed separation	
IEM resulting in direct inhibition of the urea cycle	Urea cycle disorders	Primary enzyme defects: N-acetylglutamate synthetase deficiency; carbomylphosphate synthase deficiency; ornithine transcarbamylase deficiency; argininosuccinate synthase deficiency; argininosuccinate lyase deficiency; arginase deficiency.
		Transporter defects: citrin deficiency; hyperornithinaemia hyperammmonaemia homocitrullinuria (HHH); lysinuric protein intolerance.
	Organic acidaemias	Propionic acidaemia; methylmalonic acidaemia; isovaleric acidaemia.
	Fatty acid oxidation disorders	Especially CPT1/LCHAD/VLCAD/glutaric aciduria type II.
	Other mainly	Ornithine aminotransferase deficiency (OAT); hyperinsulinism hyperammonaemia.
Hepatic dysfunction	Prehepatic	Abernathey malformation; Budd–Chiari syndrome.
	hepatic	Any cause of hepatic failure.
	Post hepatic	Biliary atresia.

CPT1, carnitine palmitoyltransferase-1 (CPT-1) deficiency; IEM, inborn errors of metabolism; LCHAD, long chain 3-hydroxyacyl-CoA dehdrogenase deficiency; VLCAD, very long chain acyl-CoA dehydrogenase deficiency.

often mimic sepsis with lethargy, irritability, temperature instability and feeding difficulties. As the ammonia level increases, respiratory distress, vomiting, alteration in tone, convulsions and apnoeas become more prominent.[13] The rate of this decline is dependent on the nature of the underlying defect and its severity: the early manifesting urea cycle disorders (UCDs) and organic acidaemias often present between 12 and 72 h of age after an initial symptom-free period.[14] However, hyperammonaemia can present at any age and the length of the classical symptom-free period may be absent or so short that it is not obvious. It is also to be noted that a respiratory alkalosis should always raise suspicion for hyperammonaemia though again it may be subtle. However, very often, the hyperammonaemic sick neonate presents with an apparent metabolic acidosis, secondary to a concurrent impairment in cardiovascular stability.

It seems sensible to target any neonate with profound encephalopathy or a pattern of presentation that does not fit with the presumed diagnosis: for example, patients with suspected hypoxic–ischaemic encephalopathy with normal Apgar scores or those with suspected sepsis but normal inflammatory markers and negative cultures.[15] Although hyperammonaemia is a relatively rare cause of neonatal illness, it should not be missed, given that it is reversible and can have detrimental effects on the long-term prognosis of affected individuals. Therefore, the authors feel that ammonia should be investigated in any neonate with unexplained non-specific systemic illness with neurological impairment. This is while fully accepting that occasionally the ammonia will have to be repeated to differentiate between false positives and those truly affected.

Infants and older children

In infants and older children the symptoms can be less acute, with some children showing a pattern of chronic failure to thrive, anorexia, lethargy and/or poor global development. Presentation can also be episodic, with neurological manifestations varying from mild behavioural disturbances and headaches to more severe symptoms such as ataxia, hemiplegia and convulsions[16]: One common clue often elicited in the history, is a self-restricted diet with the avoidance of high protein foods such as meat, cheese and fish.[17] The severity of the symptoms is a reflection of the underlying disorder and the degree of catabolic stress: a good example is a previously well woman presenting postpartum with encephalopathy, a well recognised presentation of an ornithine trancarbamylase carrier where the protein load secondary to uterine involution may result in hyperammonaemia.[18]

Because of the broad variation of clinical presentations, decision towards testing for hyperammonaemia needs to be decided by the individual case. However in the authors' opinion, plasma ammonia testing should be considered in any patient with recurrent neurological symptomatology or a degree of neurological impairment, out of keeping with their clinical history or in the absence of other explanations.

Box 1 Factors influencing serum ammonia levels

- Primary diagnosis, its severity and nature of the underlying defect. A patient with arginase deficiency will tend to manifest a lower level of hyperammonaemia than a patient with citrullinaemia. However, even within a particular disorder, the extent of hyperammonaemia is variable. This variability normally reflects the severity of underlying enzymatic impairment, with the disorders that present in the neonatal period tending to be more severe than those that present later in life.
- Amount of protein and energy the patient has been receiving in the diet prior to presentation. Inadequate dietary energy or protein provision has the potential to push the patient into a catabolic state. Inadequate provision of essential amino acids and energy drives deamination of the patients' muscle protein and increases flux through the urea cycle. The converse is also unfortunately true where excessive protein load, for example, a high protein intake can result in excessive strain on the compromised pathway. Thus, one of the key principles of long-term treatment is the provision of a safe dietary protein minimum to meet the individual's needs but minimising the flux through the urea cycle. Protein intake should be discontinued, when patients are acutely unwell and adequate calorie intake should be provided by carbohydrates. Total protein exclusion beyond 48 h will, however, result in catabolism and thus increase flux through the urea cycle.
- Patient's medication may also have a profound effect on the level of ammonia; for example, sodium valporate seems to act by reducing the amount of N-acetylglutamate, causing a secondary inhibition of carbamylphosphate synthetase, and therefore should be avoided.
- Level of muscle activity. This is the basis of exercise provocation testing where high-intensity exercise results in ammonia production from the purine nucleotide breakdown, while in prolonged submaximal exercise, ammonia production comes from the breakdown of branched chain amino acids. However. given that provocation testing traditionally requires 2 min of vigorous ischaemic exercise, this is not routinely of practical significant.

Patients with known IEM and risk of hyperammonaemia

In any patient with an urea cycle defect, repeated ammonia levels are one of the key determinants of the efficacy of long-term treatment of the low-protein diet and ammonia scavenger drugs, sodium benzoate and sodium phenylbuturate.

Additionally, ammonia levels should be among the initial investigations in any circumstances of suspected metabolic decompensation in any patient with an increased risk of hyperammonaemia (table 2). Children might have already become symptomatic, before an appreciable change in plasma ammonia is detectable. In these situations, careful observation and discussion of patients with the local metabolic team is warranted even in the face of normal ammonia levels.

The importance of the evaluation of potential hyperammonaemia cannot be overstated in these patients, as hyperammonaemia is a treatable medical emergency. The basic concepts of therapy are based on the immediate elimination of protein (reducing flux of toxic metabolites), provision of high carbohydrates to stop catabolism/promote anabolism and the use of ammonia scavenger drugs.

What is a significant level of hyperammonaemia and when is it diagnostically relevant?

The normal values for ammonia probably do not vary greatly with age of patient. Factors influencing serum ammonia levels are outlined in box 1.In term neonates typical plasma values are less that 65 μmol/l.[19] However, any sick neonate, even those without a primary metabolic disorder may have much higher values.[20] Given ammonia's capacity to rise with generalised illness, particularly in the neonatal period and the difficulties of sampling, ammonia values of >150 μmol/l in sick premature neonates, >100 μmol/l in term neonates and >40 μmol/l in older infants and children should be considered as potentially worth investigating.[21 22] The current advice from The BIMDG is that ammonia measurement must be repeated immediately in children with levels >150 μmol/l and in neonates with levels >200 μmol/l.[23]

Although historically it has been felt that the degree of hyperammonaemia and its rapidity of increase might be a guide towards underlying diagnosis, practically the value of the ammonia is not often diagnostically useful. While values of greater than 200 μmol/L are usually considered to be suggestive of a potential metabolic disorder,[2] very high levels might primarily raise the suspicion for a UCD. However, overwhelming hyperammonaemia can also be seen in organic acidaemias especially in propionic aciduria,[24] and even sepsis can result in ammonia levels of >700 μmol/l.[25] High ammonia levels are much less commonly seen in fatty acid oxidation disorder. However, similar lower levels of ammonia are seen early into decompensation in patients' diagnosed with UCDs and organic acidaemias.

One important situation where the level of ammonia may be helpful is with the diagnosis is of transient hyperammonaemia of the newborn (THAN), a condition caused by failure of closure of the ductus venous and lack of filtration of the ammonia from the blood: here, the levels of ammonia are extremely high within the first 24 h of life (often >1500 μmol/l). These levels when coupled with the history of a sick premature, often slightly growth-restricted neonate, are strongly suggestive of the diagnosis.[26] Given the relatively high survival and good neurological outcomes of THAN with aggressive treatment, this is an important differential to consider; however it is still crucial to perform a full differential investigation.

Does the degree of hyperammonaemia give a guide to prognosis?

Both survival rates and neurological outcome are affected by the duration and the degree of hyperammonaemia. Enns *et al*, investigating neonatal hyperammonaemia secondary to UCD, found that survival was related to peak ammonia level. This study showed

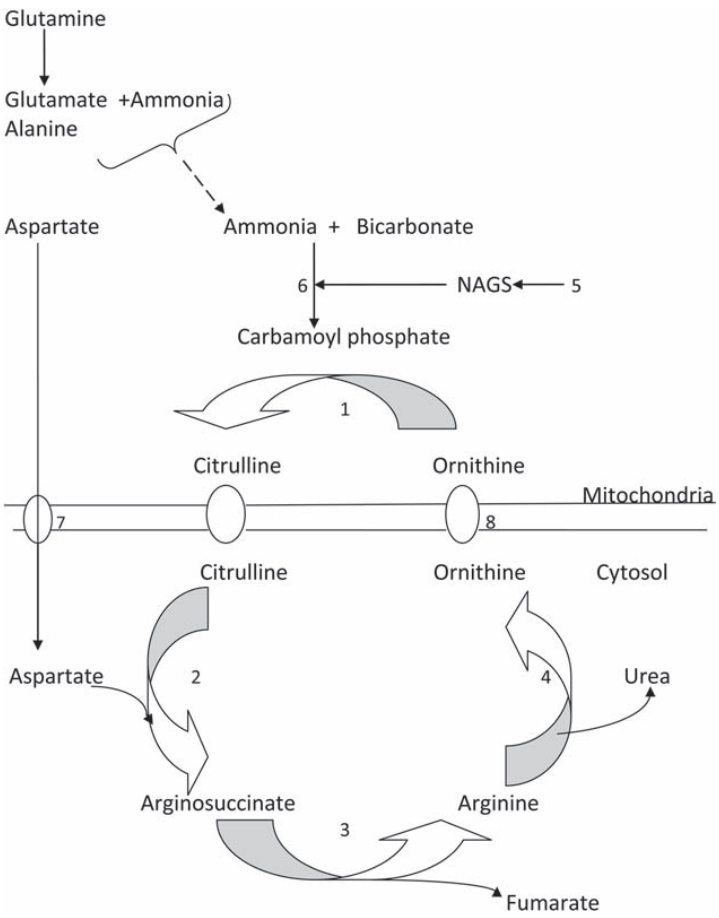

Figure 1 The urea cycle and primary urea cycle defects. Enzymes: 1, ornithine transcarbamylase; 2, argininosuccinate synthetase; 3, argininosuccinate lyase; 4, arginase; 5, N-acetylglutamate synthetase and 6, carbamyl phosphate synthetase. Transporters: 7, citrin (mitochondrial aspartate-glutamate carrier) and 8, mitochondrial ornithine transporter cause of HHH, hyperammonaemia–hyperornithaemia–homocitrullinuria.

that those patients whose maximal ammonia level was <500 μmol/l had a 94% survival rate but this fell to only 38% in those with ammonia levels >1000 μmol/l.[27] Bachmann, in a study looking at neurological outcome in 88 patients with UCDs, reported that no patient whose peak ammonia had been >480 μmol/l had a normal neurological outcome. However, it is to be noted that for the most part this study predated the regular use of ammonia scavenger drugs and modern intensive care unit practices.[28] Other studies have indicated that the duration of the encephalopathic coma is a better predictor of overall neurological outcome with increasingly poor results are seen with comas lasting greater than 48 h.[29] Finally, the underlying cause of the hyperammonaemia has also been seen to be a determinant of outcome.[30]

The meaning of routine biochemistry in the context of hyperammonaemia

Although it is beyond the scope of this study to review all biochemical changes associated with hyperammonaemia, table 1 relates to changes in other routinely available biochemical investigations. These should be taken in any child being evaluated for potential hyperammonaemia where the diagnosis is uncertain.

Limitations

The challenge in ammonia testing is the large number of false positives that are generated by poor sampling and handling of specimens, most commonly due to a delay in transit to the laboratory. This has been shown to reduce the positive predictive value of ammonia down to as less as 60%.[1] The recommendations from the UK National Metabolic Biochemistry Network state that ammonia should be measured as a free-flowing venous sample, with the avoidance of capillary samples, often difficult in a neonate, and should arrive at laboratory within 15 min, ideally on ice.[22] The reasons for these suggestions are that haemolysis secondary to capillary sampling results in increased interference in spectroscopic assay.[11 31] The rapid

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