UK Metabolic Biochemistry Network Recommendations For The Investigation of Hyperammonaemia

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Disclaimer: These are laboratory guidelines reflecting current best practice in specialist metabolic laboratories the UK. They are not evidence based but reflect expert opinion. MetBioNet cannot accept any responsibility for use of these guidelines.

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Introduction

Hyperammonaemia results from defective catabolism of amino acids to urea. Recognition and treatment of hyperammonaemia, especially in the neonatal period, is a clinical emergency as if left untreated morbidity and mortality is high. These guidelines describe the differential diagnosis of hyperammonaemia, providing guidance to the non-specialist clinician and laboratories on appropriate investigation, particularly with regards to inherited metabolic disorders. Secondly laboratories have vital role in identifying patients with hyperammonaemia at the earliest opportunity so that patients are be managed appropriately. These guidelines also provide recommendations to non-specialist clinicians and laboratories staff on the pre-analytical, analytical and post analytical aspects of ammonia measurement.

Background

Ammonia is produced from the deamination of amino acids in the liver, muscle and kidney, and by the action of gut bacteria.

$$\text{Glutamine} \rightarrow \text{Glutamate} \rightarrow \text{Oxoglutarate} + NH_4+$$

At physiological pH 95% is in the ammonium form (NH_4+) which is less permeable to cell membranes however an increase in pH results in a shift in the equilibrium towards ammonia (NH_3) enhancing toxicity. In the liver ammonia is converted to urea, via the urea cycle, for excretion by the kidneys. Most clinical chemistry methods measure total NH_3 and NH_4+. Typical plasma ammonia levels are shown in table 1.

	Ammonia
Premature Neonate	< 150 µmol/L
Term Neonate	< 100 µmol/L
Infant / Child	< 50 µmol/L
Adult	< 50 µmol/L

Table 1. Typical plasma ammonia levels

Mildly raised levels up to 80 µmol/L are seen quite commonly, and in neonates any illness may result in levels around 150 µmol/L**. Any ammonia >150 µmol/L in children, or >200 µmol/L in neonates and >100 µmol/L in adults requires immediate attention.**

Ammonia is neurotoxic and symptoms are therefore essentially neurological, however there is a wide clinical spectrum with varying severity and age of onset. Neonates with inherited metabolic disorders resulting in hyperammonaemia may have overwhelming illness (often mistaken for sepsis) with rapid deterioration from poor feeding and vomiting to tachypnoea,

convulsions and coma. Respiratory alkalosis is an early sign. There should be a low threshold for suspicion of hyperammonaemia in any neonate with neurological deterioration for no apparent cause. Patients may die during an acute episode due to cerebral oedema and those who survive the crisis often have remaining handicap or neurological deficit. In contrast to other urea cycle disorders argininaemia presents with spasticity rather than an acute hyperammonaemia syndrome. Milder defects may not present until later in life or during intercurrent illness. Symptoms of chronic hyperammonaemia may be episodic and are non-specific such as vomiting, faddy eating, behavioural changes, slow developmental progress and neurological deficits. Consequently hyperammonaemia may be difficult to recognise.

Untreated hyperammonaemia can cause irreversible brain damage and death at any age. Therefore it is essential to measure ammonia in every sick patient in which a metabolic disease may be the underlying diagnosis.

It is vital that once hyperammonaemia is suspected that a blood sample is obtained, analysed and reported as quickly as possible. Delays in analysis and reporting of hyperammonaemia have contributed to significant patient harm as described in two recent Patient Safety Bulletins.

Causes of Hyperammonaemia

The causes of hyperammonaemia can be classified as detailed in table 2.

DEFECTS OF THE UREA CYCLE	OTHER METABOLIC DISORDERS
Inherited Deficiencies of : - N-Acetyl glutamate synthase (NAGS) - Carbamoyl phosphate synthase (CPS) - Ornithine transcarbamylase (OTC) - Argininosuccinate synthase (citrullinaemia) - Argininosuccinate lyase (argininosuccinic aciduria) - Arginase (argininaemia) - Carbonic anhydrase VA deficiency	**Organic Acidurias** e.g Propionic aciduria, Methylmalonic aciduria, Isovaleric aciduria **Disorders of Fatty Acid Oxidation** e.g Medium chain acyl-CoA dehydrogenase deficiency Carnitine palmitoyltransferase II deficiency **Others** - HHH syndrome - Lysinuric protein intolerance (LPI) - Hyperinsulinaemic hyperammonaemia - Ornithine aminotransferase deficiency (OAT) (neonatal form) - Mitochondrial respiratory chain defects - Pyruvate dehydrogenase deficiency - Citrin deficiency (citrullinaemia type II) - Congenital lactic acidosis
ACQUIRED	**OTHER**
• Liver failure / impairment • Urinary tract infection • GI bacterial overgrowth • Drugs e.g. valproate, chemotherapy • Total parenteral nutrition • Severe illness e.g. asphyxia, sepsis • Reyes syndrome • Systemic herpes simplex	• Artefactual increase : - poor specimen quality / haemolysis - difficult venepuncture - skin contamination - contaminated tube - delayed analysis / protein breakdown • Transient hyperammonaemia of the Newborn

Table 2. Causes of Hyperammonaemia

PART 1

Investigation of Hyperammonaemia

1. Check Ammonia

Ideally ammonia should be measured on a free-flowing venous sample or arterial stab. Capillary samples should be avoided. Samples should be sent to the laboratory as soon as possible (ideally within 15 minutes and on ice). However if it is not possible to collect the 'perfect sample' and send under ideal conditions, send the sample anyway. If a high ammonia result is obtained, the result should be confirmed by repeat analysis as soon as possible. An increasing trend in the ammonia concentration provides evidence towards a metabolic cause, with particularly rapid increases being seen in urea cycle disorders.

The commonest cause of a mildly raised ammonia level is contamination or sample deterioration. If it is suspected that the raised ammonia is due to artefactual factors, the result should be confirmed on a second sample prior to initiating treatment.

2. Ammonia Level

The ammonia level may assist in the different diagnosis of the cause of hyperammonaemia. **In general an ammonia level of greater than 200 μmol/L is more likely to have a metabolic cause whereas a level of less than 200 μmol/L is more likely to be acquired.** The highest levels of ammonia are usually seen in urea cycle disorders such as OTC, CPS and NAGS, the acute organic acidurias, such as propionic acidaemia, and transient hyperammonaemia of the newborn. Metabolic conditions such as fatty acid oxidation disorders, hyperinsulinaemic hyperammonaemia and ornithine aminotransferase deficiency tend to have more moderate increases in ammonia. Some metabolic conditions, such as medium chain acyl-CoA dehydrogenase deficiency, mitochondrial disorders may present with normal or mildly raised ammonia levels.

3. First Line Investigations

First line investigations all patients with hyperammonaemia should include:

Blood	Blood gases Urea and electrolytes Liver function tests Clotting studies Glucose Lactate Calcium Culture	**Blood or urine**	Ketones

Results of these investigations may support an acquired cause or provide evidence for an underlying metabolic condition, directing further investigations. Findings associated with particular metabolic conditions are detailed in table 3.

Investigation	Interpretation
Blood Gases	Ammonia is a respiratory stimulant therefore hyperammonaemia causes a **respiratory alkalosis**. The presence of a **metabolic acidosis** may suggest an organic acid disorder or fatty acid oxidation defect.
Urea	May be inappropriately **low** compared to other markers of renal function / dehydration in urea cycle disorder.
Liver Function Tests	**Severely deranged** in some acquired causes of hyperammonaemia. May be **mild elevations** in urea cycle defects and organic acidurias.
Glucose	**Hypoglycaemia** may occur in e.g. fatty acid oxidation disorders, hyperinsulinism and liver failure. (See MetBioNet guidelines for investigation)
Lactate	May be **raised** in a number of metabolic conditions and also liver failure. (See MetBioNet guidelines for investigation)
Calcium	**Hypocalcaemia** is a feature of some organic acid disorders.
Ketones	May differentiate organic acids disorders **(increased)** from fatty acid oxidation disorders and liver failure **(not present/low).**

Table 3. First Line Biochemical Investigations in Hyperammonaemia

4. Specialist Investigations

The following specialist metabolic investigations should be carried where a metabolic cause of hyperammonaemia is suspected:

- **Plasma and urine amino acids**
- **Urine organic acids including orotic acid**
- **Blood spot or plasma acylcarnitines**

Ideally samples should be collected prior to the initiation of treatment.

Plasma amino acids should be done **urgently** if there is significant hyperammonaemia and a urea cycle disorder is suspected.

The results of these investigations are usually diagnostic, however further confirmatory tests may be required.

Table 4 highlights the role of **urine orotic acid** and **plasma amino acids** in the differential diagnosis of urea cycle disorders indicating the potential findings. Citrullinaemia, argininosuccinic aciduria and argininaemia can all be diagnosed on the basis of amino acid results. Glutamine, glutamate, asparagine, aspartate, lysine and alanine can all be high and arginine may be low as non-specific findings secondary to hyperammonaemia.

Disorder	Urine orotic acid	Plasma amino acids
N-acetyl glutamate synthase deficiency	N or ↓	Citrulline ↓ Arginine ↓
Carbamoyl phosphate synthase deficiency	N or ↓	Citrulline N or ↓ Arginine N or ↓
Ornithine transcarbamylase deficiency	↑↑	Citrulline ↓ Arginine ↓ Lysine ↑
Citrullinaemia	↑	Citrulline ↑↑ Arginine ↓
Argininosuccinic aciduria	↑	Argininosuccinate ↑↑ Citrulline ↑ Arginine ↓
Argininaemia	↑↑	Arginine ↑↑

N = Normal ↑= Increased ↓= Decreased

Table 4. Differential Diagnosis of Urea Cycle Disorders

Urine organic acid analysis is diagnostic of organic acid disorders, each of which results in excretion of characteristic metabolites. In fatty acid oxidation disorders a dicarboxylic aciduria may be seen.

Acylcarnitines are diagnostic of fatty acid oxidation disorders which are differentiated according to the pattern of elevated acylcarnitines. Some acyl- carnitines and other carnitine conjugates are elevated in organic acid disorders.

5. Confirmatory Tests

Genetic and enzyme studies are available for the confirmation of many of the disorders resulting in hyperammonaemia. Enzyme studies are carried out on liver, fibroblasts or erythrocytes depending on the condition. Further details are available via the MetBioNet Metabolic Assays Directory (www.metbio.net) and the UK Genetics Testing Network (www.ukgtn.org).

6. Management of Hyperammonaemia

Please refer to the BIMDG: 'Emergency Protocols for Undiagnosed Hyperammonaemia and Immediate Management'.

PART 2

Guidance for the Measurement of Ammonia in Blood / Plasma

All hospitals with labour wards, neonatal units, paediatric wards and accident and emergency departments should provide a robust and reliable analytical service for measuring ammonia **24 hours a day, seven days a week**.

- All laboratories performing ammonia analysis should be accredited to ISO15189 and ensure that their ammonia assay is included in their scope of accreditation.
- All laboratories should have details of precautions to be observed for sample handling, collection and transportation to the laboratory documented in their standard operating procedures (SOP).
- Laboratories should **accept all blood samples** for ammonia analysis even if the quality of the sample has been compromised.
- Laboratories should complete a risk assessment if accepting all blood samples deviates from the manufacturer's instructions.
- All staff who may be required to perform ammonia measurements should be aware of the factors contributing to artefactual increases in ammonia i.e. haemolysis, delay in analysis.
- All staff who perform ammonia analyses should be familiar with the laboratory SOP and know the operating characteristics and limitations of their assay. Training and revalidation should be provided for staff working in laboratories with a small analytical workload who undertake the analysis on an infrequent basis.

Sample Collection/Transportation/ Pre-analytical

To avoid artefactual increases in ammonia good sampling technique and rapid delivery of the sample to the laboratory is required.

- A free-flowing venous blood sample should be collected into specimen tubes containing either lithium heparin or EDTA as anticoagulant as per local laboratory instructions. Drawing blood through a small indwelling catheter may cause haemolysis and hence spuriously elevated ammonia; ideally blood obtained this way should be avoided.
- All specimens for ammonia analysis should be transported to the laboratory as soon as possible, ideally within 15 minutes of collection and preferably on wet ice.
- Laboratories should **accept all blood samples** for ammonia analysis even if the quality of the sample is less than ideal.
- The samples should be analysed immediately if the measurement is to be performed on whole blood or the plasma immediately separated and analysed. If a delay in analysis is envisaged, separated plasma can be stored for up to 4 hours at 4^{o}C.
- Laboratories should document any delay in sample receipt and whether there is significant haemolysis as these are important causes of raised ammonia.
- Environment sources of ammonia may be present in clinical areas and that vacutainers and equipment used for sample collection may be contaminated. Possible sources of contamination should be minimised.

Analytical Methodology

- Laboratories should **analyse all blood samples** for ammonia even if the quality of the sample is less than ideal.
- The majority of laboratories in the UK employ automated enzyme based (glutamate dehydrogenase) methods or dry slide chemistry for the measurement of plasma ammonia.
- Assays that include a sample blank correction step are preferred in order to avoid assay interference especially in patients with acute liver failure.
- The analytical method should be carefully selected to ensure that it is fit for purpose and consider whether the analytical range is appropriate for monitoring response to treatment.
- Reflectance meters employing dry slide chemistry strips for whole blood ammonia are not recommended, due to their limited measuring range of up to **285 µmol/L.** They are **not** suitable for the monitoring of hyperammonaemia patients in whom treatment decisions require knowledge of the absolute concentration. A careful risk

assessment should be completed if such meters are being considered/used so that the risks can be mitigated.

- All potential extraneous sources of ammonia contamination from other reagents (i.e. urease containing solutions) and water supply should be minimised,
- All laboratories should measure and record the haemolysis index (and icterus index if their assay is affected) of all plasma samples for ammonia analysis.

Quality Control and Quality Assurance

- All laboratories must quality control their ammonia analyses using third party quality control material.
- Laboratories should participate in an External Quality Assurance scheme e.g WEQAS

Reporting/Reference Intervals

- All laboratories should have agreed age related reference ranges for neonates (up to the age of 1 month), infants, children and adults. These reference ranges should be appropriate for the analytical method employed i.e. enzymatic, whole blood reflectance measurements etc.
- Laboratories should **append appropriate comments** to all ammonia results where samples have been delayed or are haemolysed or otherwise compromised.
- Laboratories should request a repeat sample for confirmation of hyperammonaemia where the ammonia result is elevated for the first time.
- Ammonia results of >100 µmol/L should be communicated urgently to the requesting doctor/clinical team.

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Definitions

CPS	Carbamoyl phosphate synthase
HHH	Hyperammonaemia, Hyperornithinaemia, Homocitrullinaemia
LPI	Lysinuric protein intolerance
MetBioNet	Metabolic Biochemistry Network
NAGS	N-Acetyl glutamate synthase
OAT	Ornithine aminotransferase
OTC	Ornithine transcarbamylase

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