UK NATIONAL METABOLIC BIOCHEMISTRY NETWORK

GUIDELINES FOR THE INVESTIGATION OF HYPERAMMONAEMIA

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 / laboratories on appropriate investigation, particularly with regards to inherited metabolic disorders.

INTRODUCTION

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

Ammonia → Glutamine → Glutamate → Oxoglutarate + NH₄⁺

At physiological pH 95% is in the ammonium form (NH₄⁺) which is less permeable to cell membranes however an increase in pH results in a shift in the equilibrium towards ammonia (NH₃) 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 NH₃ and NH₄⁺. Normal plasma ammonia levels are shown in table 1.

Table 1 – Normal ranges for plasma ammonia.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premature Neonate</td>
<td>&lt; 150 μmol/L</td>
</tr>
<tr>
<td>Term Neonate</td>
<td>&lt; 100 μmol/L</td>
</tr>
<tr>
<td>Infant / Child</td>
<td>&lt; 40 μmol/L</td>
</tr>
</tbody>
</table>

Measurement of ammonia should be one of the first line biochemical investigations undertaken in the acutely ill neonate or young infant.

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. 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. Milder defects may not present until later in life or during intercurrent illness. In some cases symptoms may be episodic. Symptoms of chronic hyperammonaemia include vomiting, faddy eating, behavioural changes, slow developmental progress and neurological deficits.

CAUSES OF HYPERAMMONAEMIA

The causes of hyperammonaemia can be classified as detailed in table 2.
Table 2 – Causes of Hyperammonaemia

<table>
<thead>
<tr>
<th>DEFECTS OF THE UREA CYCLE</th>
<th>OTHER METABOLIC DISORDERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Inherited Deficiencies of :</td>
<td>• Organic Acidurias</td>
</tr>
<tr>
<td>- N-Acetyl Glutamate Synthase (NAGS)</td>
<td>• Disorders of Fatty Acid Oxidation</td>
</tr>
<tr>
<td>- Carbamoyl Phosphate Synthase (CPS)</td>
<td>• Others :</td>
</tr>
<tr>
<td>- Ornithine Transcarbamylase (OTC)</td>
<td>- HHH Syndrome</td>
</tr>
<tr>
<td>- Argininosuccinate Synthase (Citrullinaemia)</td>
<td>- Lysinuric Protein Intolerance</td>
</tr>
<tr>
<td>- Argininosuccinate Lyase (Argininosuccinic Aciduria)</td>
<td>- Hyperinsulinaemic Hyperammonaemia</td>
</tr>
<tr>
<td>- Arginase (Argininaemia)</td>
<td>- Ornithine Aminotransferase Deficiency (neonatal form)</td>
</tr>
<tr>
<td>• Organic Acidurias</td>
<td>- Mitochondrial Respiratory Chain Defects</td>
</tr>
<tr>
<td>• Disorders of Fatty Acid Oxidation</td>
<td>- Pyruvate Dehydrogenase Deficiency</td>
</tr>
<tr>
<td>• Others :</td>
<td>- Citrin Deficiency (Citrullinaemia Type II)</td>
</tr>
<tr>
<td>- HHH Syndrome</td>
<td>• Artefactual Increase :</td>
</tr>
<tr>
<td>- Lysinuric Protein Intolerance</td>
<td>- poor specimen quality / haemolysis</td>
</tr>
<tr>
<td>- Hyperinsulinaemic Hyperammonaemia</td>
<td>- difficult venepuncture</td>
</tr>
<tr>
<td>- Ornithine Aminotransferase Deficiency (neonatal form)</td>
<td>- skin contamination</td>
</tr>
<tr>
<td>- Mitochondrial Respiratory Chain Defects</td>
<td>- contaminated tube</td>
</tr>
<tr>
<td>- Pyruvate Dehydrogenase Deficiency</td>
<td>- delayed analysis</td>
</tr>
<tr>
<td>- Citrin Deficiency (Citrullinaemia Type II)</td>
<td>• Transient Hyperammonaemia of the Newborn</td>
</tr>
</tbody>
</table>

INVESTIGATION

1. Check Ammonia

The commonest cause of a raised plasma ammonia level is contamination or sample deterioration. Prior to initiating treatment all elevated ammonia results should be confirmed on a second sample to exclude artefactual increases.

Ammonia should be measured on a free flowing venous sample or arterial stab. Capillary samples should be avoided. Samples should be sent to the lab within 15 minutes, ideally on ice.

If a high ammonia is confirmed analysis should be repeated within 4 hours. An increasing trend in the ammonia concentration provides evidence towards a metabolic cause, with particularly rapid increases being seen in urea cycle disorders.
2. Ammonia Level

The ammonia level may assist in the different diagnosis of the cause of hyperammonaemia. In general an ammonia of < 200 µmol/L is more likely to be acquired whereas a level > 200 µmol/L suggests a metabolic cause. Higher levels of ammonia are seen in urea cycle disorders and organic acidurias than in metabolic disorders causing secondary increases in ammonia. Table 3 indicates the ammonia levels which may be expected in different conditions. Relating the level of ammonia to likely diagnosis should be done with a degree of caution, and used as a guide only, as varying levels have been seen in different conditions.

Table 3 – Expected Elevations in Ammonia

<table>
<thead>
<tr>
<th>Ammonia (µmol/L)</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 1500</td>
<td>Transient Hyperammonamia of the Newborn</td>
</tr>
</tbody>
</table>
| > 600            | Urea Cycle Disorders  
|                  | Propionic Acidaemia  
|                  | Multiple Acyl CoA Dehydrogenase Deficiency  
|                  | HHH Syndrome  
|                  | Lysinuric Protein Intolerance  
|                  | Pyruvate Dehydrogenase Deficiency  
|                  | Reyes Syndrome  
|                  | Sodium Valproate Therapy |
| 200 – 600        | Organic Acidurias  
|                  | Fatty Acid Oxidation Disorders  
|                  | Hyperinsulinaemic Hyperammonaemia  
|                  | Ornithine Aminotransferase Deficiency |
| < 200            | Acquired  
|                  | e.g. illness/sepsis, TPN, chemotherapy, liver dysfunction.  
|                  | Some Metabolic Conditions  
|                  | e.g. MCADD,organic acidaemias, mitochondrial disorders |

3. First Line Investigations

First line investigations all patients with hyperammonaemia should include:

**Blood**  
Blood Gases  
Urea and Electrolytes  
Liver Function Tests

**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 4.

*Table 4 – First Line Biochemical Investigations in Hyperammonaemia*

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

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

- Urine and Plasma Amino Acids
- Urine Organic Acids
- Urine Orotic Acid
- Blood Spot or Plasma Acyl Carnitines

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

Table 5 highlights the role of *urine orotic acid* and *plasma amino acids* in the differential diagnosis of urea cycle disorders indicating the potential findings. Citrullinaemia, Arginosuccinic Aciduria and Argininamia can all be diagnosed on the basis of amino acid results. Glutamine, Alanine and Asparagine may be non-specifically increased in all urea cycle disorders.

**Table 5 – Differential Diagnosis of Urea Cycle Disorders**

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Urine Orotic Acid</th>
<th>Plasma Amino Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Acetyl Glutamate Synthase Deficiency</td>
<td>N or ↓</td>
<td>Citrulline ↓ Arginine ↓</td>
</tr>
<tr>
<td>Carbamoyl Phosphate Synthase Deficiency</td>
<td>N or ↓</td>
<td>Citrulline N or ↓ Arginine N or ↓</td>
</tr>
<tr>
<td>Ornithine Transcarbamylase Deficiency</td>
<td>↑↑</td>
<td>Citrulline ↓ Arginine ↓ Lysine ↑</td>
</tr>
<tr>
<td>Citrullinaemia</td>
<td>↑</td>
<td>Citrulline ↑↑ Arginine ↓</td>
</tr>
<tr>
<td>Argininosuccinic Aciduria</td>
<td>↑</td>
<td>Argininosuccinate ↑↑ Citrulline ↑ Arginine ↓</td>
</tr>
<tr>
<td>Argininaemia</td>
<td>↑↑</td>
<td>Arginine ↑↑</td>
</tr>
</tbody>
</table>

N = Normal  ↑= Increased  ↓= Decreased
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.

Acyl Carnitines 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

Enzyme and/or DNA 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).

REFERENCES


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Version: 2

These are laboratory guidelines reflecting current best practice in specialist metabolic laboratories in the UK. The network cannot accept any responsibility for the use of these guidelines.
DIAGNOSTIC ALGORITHM

? Hyperammonaemia

Plasma ammonia > 100 µmol/L (neonate) of > 40 µmol/L (infant / child)

CONFIRM BY REPEAT SAMPLING

Ammonia < 200 µmol/L

? Acquired

Ammonia > 200 µmol/L

? Metabolic

FIRST LINE BIOCHEMICAL INVESTIGATIONS
(for interpretation see table 4)

- Blood Gases
- Urea and Electrolytes
- Liver Function Tests
- Clotting Studies
- Glucose
- Lactate
- Calcium
- Urine Ketones

Further Specialist Investigations if Metabolic Cause Suspected

? OA or FAO Disorder
e.g. acidosis ± ketones

Urine Organic Acids
Blood Spot Acyl Carnitines

? Urea Cycle Disorder
e.g. respiratory alkalosis

Urine and Plasma Amino Acids
Urine Orotic Acid

? Other Metabolic Cause
(when other causes excluded)
e.g. Amino Acids, Insulin, PDH

Confirmatory Tests if Required

APPENDIX
Notes on the Measurement of Ammonia in Blood / Plasma

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

- All laboratories should have details of precautions to be observed for sample handling and collection and the maximum permissible time for the sample in transit to the lab documented in their standard operating procedure.
- 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 of the analysis i.e. limit of linearity, any instrument error codes etc. 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
The most common cause of raised ammonia is artefactual and pre-analytical in origin due either to poor sample collection or delay in analysis.

- A free-flowing venous blood sample should be collected into specimen tubes containing either lithium heparin or EDTA as anticoagulant and which have been determined to be free of ammonia contamination. Drawing blood through a small indwelling catheter may cause haemolysis and hence spuriously elevated ammonia; ideally blood obtained this was should be avoided (1).
- All specimens for ammonia analysis should be transported to the laboratory within 15 minutes of collection, ideally on ice, and the samples analysed immediately if the measurement is to be performed on whole blood or the plasma immediately separated. If a delay is envisaged separated plasma can be stored for up to 4 hours at 4°C (2).
- It is important to document whether there is significant haemolysis as this is an important cause of raised ammonia.

Analytical Methodology

- The majority of laboratories in the UK employ automated enzyme based (glutamate dehydrogenase) methods or dry slide chemistry for the measurement of plasma ammonia. Reflectance meters employing dry slide chemistry strips for whole blood ammonia are suitable for the initial SCREENING of patients for hyperammonaemia and as such are a suitable alternative for laboratories with a small workload. All abnormal specimens should be confirmed by a quantitative method.
  **Caution:**
  However the working range of meters is limited to 285 µmol/L. They are therefore **NOT** suitable for the monitoring of hyperammonaemia patients in whom treatment decisions require knowledge of the absolute concentration.

- All potential extraneous sources of ammonia contamination from other reagents (i.e. urease containing solutions) and water supply should be minimised,
• Laboratory staff should comment appropriately if they suspect an elevated result is due to artefactual contribution i.e. haemolysis, delay in analysis.

Quality Control and Quality Assurance

• All laboratories must quality control their ammonia analyses using either commercially available material or solutions of ammonium salts made up in-house.
• Where available laboratories should participate in External Quality Assurance.

Reference Ranges

• All laboratories should have agreed age related reference ranges for neonates (up to the age of 1 month), and infants, children and adults. These reference ranges should be appropriate for the analytical method employed i.e. enzymatic, whole blood reflectance measurements etc.

References


(Appendix written by Dr Jim Bonham, Sheffield Children’s Hospital)