UK National Metabolic Biochemistry Network

Guidelines for the Investigation of Hyperammonaemia for Inherited Metabolic Disorders
Aims

To provide guidance for non-specialist laboratories on the investigation of hyperammonaemia for possible inherited metabolic disorders (IMD) - particularly in the acutely ill neonate or infant.

What is hyperammonaemia?

Ammonia is produced principally from the catabolism of amino acids. In normal circumstances ammonia is converted to urea by the urea cycle and plasma concentrations are maintained at low levels. Hyperammonaemia is an excessive concentration of circulating ammonia caused by disrupted functioning of the urea cycle. The reference intervals for plasma ammonia are age dependent:

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Reference Interval</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 &amp; child</td>
<td>&lt;40 µmol/L</td>
</tr>
</tbody>
</table>

Causes of hyperammonaemia

Hyperammonaemia may be due to the following:-

- Pre analytical factors
- Inherited Defects of the Urea Cycle (Table 1)
- Other Inherited Metabolic Disorders (Table 1)
- Acquired (Table 2)

The most common cause of raised plasma ammonia is artefactual due to poor sample collection or a delay in analysis. Hyperammonaemia can be caused by inherited deficiencies of the enzymes of the urea cycle. They are individually rare disorders but have a combined estimated incidence of approximately 1:30,000. The commonest disorder is ornithine transcarbamylase deficiency (OTC).

It can also occur secondary to other inherited metabolic defects which compromise the normal functioning of the urea cycle e.g. defects in organic acid metabolism.

In addition to inherited defects in metabolism, acquired hyperammonaemia can occur due to a variety of other causes including hepatic and/or other organ dysfunction.

When to suspect hyperammonaemia? / Clinical Presentation

Ammonia is neurotoxic; therefore the principal clinical features are neurological. There is a spectrum of clinical presentation which ranges from an acutely presenting, catastrophic illness in the newborn period to a more insidious, less severe and episodic clinical course in older infants, children and even adults.

The age and severity of the clinical presentation is associated with the severity of the metabolic defect.
The recognition of hyperammonaemia especially in the neonatal period is a clinical **emergency** as if untreated, morbidity and mortality are high.

**Neonatal presentations:**

Neonates presenting with inherited defects in the urea cycle usually have an initial 24-48 hour period of well being after which the clinical features associated with hyperammonaemia become apparent. The initial clinical deterioration is often mistaken for sepsis as the features of feeding difficulties and lethargy are non-specific. If untreated the neurological status progressively worsens with the development of vomiting, convulsions and coma.

### Acute Hyperammonaemia

- tachypnoea
- lethargy
- vomiting
- convulsions
- encephalopathy

Increasing

blood

Ammonia

Concentration

**Later infancy, childhood and adulthood**

Infants with less severe enzyme deficiencies usually present after the neonatal period with non-specific features including developmental delay, failure to thrive and vomiting and unexplained encephalopathy.

The presentation can also be fluctuating and episodic with mild neurological manifestations such as behavioural disturbances, headaches and vomiting or more severe coma and convulsions:

More rarely the presentation of milder defects in the urea cycle can be delayed until adolescence/adulthood and may be precipitated by an event e.g. protein load.

### Chronic Hyperammonaemia

- vomiting (may be cyclical)
- faddy eating (high protein food avoidance)
- behavioural changes
- neurological deficits (eg spastic diplegia as in arginase deficiency)

There should be a low threshold for suspicion of hyperammonaemia in any infant, particularly in the neonatal period, whose neurological status deteriorates for no apparent cause.

Measurement of ammonia should be one of the first line biochemical investigations to be undertaken in the acutely ill neonate or young infant - **see Diagnostic algorithm**.
**Diagnostic algorithm for suspected hyperammonaemia**

**CLINICAL FEATURES**

- **Neonates**: Unexplained neurological deterioration in first week of life
- **Infants/Children**: Episodic illness e.g. cyclical vomiting, unexplained neurobehavioural changes, unexplained liver disease, unexplained encephalopathy.

? **Hyperammonaemia**

**FIRST LINE BIOCHEMISTRY**

Ammonia (P)
Urea & electrolytes (P)
Blood gases
Glucose & lactate (P)
Liver function tests (P)
Urine ketones

( P) - Plasma

**Plasma Ammonia > 100 µmol/l**

**confirm by REPEAT sampling**

Exclude artefactual increases

**Ammonia > 100 µmol/l**

Exclude acquired hyperammonaemia

**AMMONIA >300 µmol/l**

Severe Hyperammonaemia
- Respiratory Alkalosis
- Low plasma Urea
Δ ? Urea Cycle defect

**AMMONIA 100-300 µmol/l**

Mild hyperammonaemia
- Metabolic acidosis
- Hypoglycaemia
- Ketonuria
- Hypocalcaemia
Δ ? Organic acid disorder

**SPECIALISED METABOLITE PROFILING**

- **Urea Cycle defect**
  - Amino acids (urine and plasma)
  - Urine orotic acid
  - Urine organic acids
- **Other IMD**
  - Amino Acids (urine & plasma)
  - Insulin
- **Organic acid disorder**
  - Urine organic acids
  - Blood spot acyl carnitines
The ammonia must be repeated as a matter of urgency to confirm the abnormal result – the most common cause of mildly increased ammonia is delay in processing of samples/poor sample collection technique i.e. artefactual. (see Appendix – Measurement of Ammonia in blood/plasma)

A normal plasma collected from a symptomatic infant excludes a Urea Cycle Defect

If the ammonia concentration is higher on repeat testing this provides additional evidence for a metabolic disorder. If the confirmed results is greater than 150 µmol it should be repeated again within 4 hours as concentration may increase rapidly if the patient has a urea cycle defect.

The degree of elevation in ammonia can assist in the differential diagnosis (see following table). Other first line investigations can help with differential diagnosis at this stage (Table 3). A raised ammonia, reduced urea and a respiratory alkalosis – together with the clinical status are suggestive of a urea cycle disorder.

<table>
<thead>
<tr>
<th>Plasma Ammonia (µmol/L)</th>
<th>Interpretation</th>
</tr>
</thead>
</table>
| <100                    | • No clinical significance in the acutely unwell neonate – see reference range  
                          • May be significant in the context of later presentations and other metabolic disorders in the infant/child |
| 100-300                 | Mild symptomatic hyperammonaemia develops at concentrations above 100 – lethargy, confusion, vomiting  
                          • Could reflect increase secondary to other metabolic disorders  
                          • Commonly observed in acquired hyperammonaemia – see Table 2 |
| 300-500                 | Significant encephalopathic features develop at concentrations above 300 – increased likelihood of urea cycle defect |
| 500-2000                | Severe hyperammonaemia associated with coma and convulsions  
                          Neonatal onset urea cycle disorders/organic acid disorders likely |

Specialised investigations to investigate hyperammonaemia

The suspicion that hyperammonaemia is due either to urea cycle defects or secondary to other metabolic disorders should prompt early contact with the regional metabolic centre to coordinate more specialised investigations and clinical management (Table 4). These specialised investigations are best undertaken at the tertiary care facility to which the child is transferred for clinical management. They need to be processed as a matter of urgency to help locate the specific enzyme defect in order to optimise management. If the condition is life threatening investigations should be according to guidelines for Sudden Unexpected Death in Infancy.
**Interpretation of specialised investigations and differential diagnosis**

Profiling of amino acids, organic acids and acyl carnitines will usually enable a presumptive diagnosis of a specific defect in the urea cycle, organic acid metabolism or fatty acid oxidation pathways. Confirmatory enzyme and/or molecular tests can be undertaken when the clinical condition has stabilised (Table 5).

**Table 1: Causes of Hyperammonaemia – Inherited Metabolic Disorders (IMD)**

<table>
<thead>
<tr>
<th>Enzyme defects of the urea cycle:</th>
<th>N-acetyl glutamate synthetase (NAGS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamyl phosphate synthetase</td>
<td></td>
</tr>
<tr>
<td>Ornithine transcarbamylase (OTC) - most common (X-linked disorder)</td>
<td></td>
</tr>
<tr>
<td>Argininosuccinate synthetase</td>
<td></td>
</tr>
<tr>
<td>Argininosuccinate lyase</td>
<td></td>
</tr>
<tr>
<td>Arginase</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Defects in organic acid metabolism:</th>
<th>Propionic acidaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylnalonic acidaemia</td>
<td></td>
</tr>
<tr>
<td>Isovaleric acidaemia</td>
<td></td>
</tr>
<tr>
<td>Hydroxymethylglutaryl CoA lyase deficiency</td>
<td></td>
</tr>
<tr>
<td>Fatty acid oxidation defects</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other IMD:</th>
<th>Hyperornithinaemia, hyperammonaemia, homocitrullinuria syndrome (HHH)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lysinuric protein intolerance</td>
</tr>
<tr>
<td></td>
<td>Hyperinsulinism hyperammonaemia syndrome</td>
</tr>
</tbody>
</table>

**Table 2: Acquired (non-IMD) causes of hyperammonaemia**

<table>
<thead>
<tr>
<th>Artefactual - preanalytical</th>
<th>Delay in analysis/ poor specimen collection/ haemolysis/ struggling infant/ specimen contamination (see Appendix)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non IMD-miscellaneous</td>
<td>Critically ill/septic infants - hypovolaemic shock Perinatal asphyxia Transient hyperammonaemia of the newborn</td>
</tr>
<tr>
<td></td>
<td>Hepatic failure Congenital intra- and extra-hepatic shunts Congestive heart failure</td>
</tr>
<tr>
<td></td>
<td>Congenital bladder defects corrected by ureterosigmoidoscopy Urinary tract infection (urease producing bacterium)</td>
</tr>
<tr>
<td></td>
<td>Gastrointestinal bacterial overgrowth - blind loop Drugs: valproate, chemotherapy Parenteral Nutrition</td>
</tr>
<tr>
<td>Reye’s Syndrome</td>
<td>Reye’s syndrome may be due to an underlying IMD and is important to investigate.</td>
</tr>
</tbody>
</table>
Table 3: First line biochemical investigations to investigate hyperammonaemia

<table>
<thead>
<tr>
<th>Test</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (plasma)</td>
<td>May be inappropriately low compared to other measures of dehydration/renal function (cf creatinine) in urea cycle disorders.</td>
</tr>
<tr>
<td>Blood gases</td>
<td>Respiratory alkalosis is a hallmark of established hyperammonaemia due to stimulation of the respiratory centre, it is rarely observed in other causes of severe neonatal illness. Conversely a primary metabolic acidosis is more a feature of organic acid disorders.</td>
</tr>
<tr>
<td>Liver function tests (plasma)</td>
<td>Usually normal in urea cycle disorders but there may be mild elevations in liver enzymes.</td>
</tr>
<tr>
<td>Sodium, potassium (plasma)</td>
<td>Not usually abnormal in urea cycle disorders.</td>
</tr>
<tr>
<td>Calcium (plasma)</td>
<td>Hypocalcaemia is a feature of organic acid disorders.</td>
</tr>
<tr>
<td>Lactate (plasma/blood)</td>
<td>May be non-specifically raised in urea cycle disorders. (see Metbionet guidelines for investigation of Lactic Acidosis)</td>
</tr>
<tr>
<td>Glucose (plasma/blood)</td>
<td>Hypoglycaemia is NOT a feature of urea cycle defects (see Metbionet guidelines for investigation of hypoglycaemia)</td>
</tr>
<tr>
<td>Urine ketones</td>
<td>Increased in disorders of organic acid metabolism.</td>
</tr>
</tbody>
</table>

Table 4: Specialised Metabolic Investigations

<table>
<thead>
<tr>
<th>Test</th>
<th>Interpretation</th>
</tr>
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</table>
| Amino acids (plasma)        | A raised glutamine (and alanine and asparagine) is a non specific feature of all urea cycle defects.  
A raised glutamine (and alanine and asparagine) is a non specific feature of all urea cycle defects.  
Citrulline is increased (X 100 normal) in argininosuccinate synthetase deficiency and (X 10 normal) in argininosuccinate lyase deficiency.  
Citrulline is reduced/ absent in NAGS/OTC and CPS deficiency. Arginine is increased (10-20X normal) in arginase deficiency and reduced in other urea cycle enzyme defects |
| Amino acids (urine)         | Diagnostic for argininosuccinic aciduria (Argininosuccinate and anhydrides), HHH, lysinuric protein intolerance                                  |
| Organic acids (urine)       | A raised orotic acid is found in some urea cycle defects. Diagnostic of organic acid and fatty acid oxidation disorders in which there is a secondary increase in ammonia. |
| Orotic acid (urine)         | In urea cycle disorders where carbamoyl phosphate (CP) accumulates there is increased production of orotic acid                                |
| Acylcarnitines (plasma/dried blood spot) | Diagnostic of fatty acid and organic acid disorders |
Table 5: Enzyme and molecular diagnostic tests for the confirmation of Urea Cycle Defects


<table>
<thead>
<tr>
<th>Enzyme Deficiency</th>
<th>Enzymology</th>
<th>Molecular Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-acetyl glutamate synthetase</td>
<td>Liver</td>
<td>+</td>
</tr>
<tr>
<td>Carbamyl phosphate synthetase</td>
<td>Liver</td>
<td>+ (linkage)</td>
</tr>
<tr>
<td>Ornithine transcarbamylase</td>
<td>Liver</td>
<td>+</td>
</tr>
<tr>
<td>Arginosuccinic acid synthetase</td>
<td>Cultured fibroblasts</td>
<td>+</td>
</tr>
<tr>
<td>Arginosuccinic acid lyase</td>
<td>Erythrocytes</td>
<td>+ (Sequencing)</td>
</tr>
<tr>
<td></td>
<td>Cultured fibroblasts</td>
<td></td>
</tr>
<tr>
<td>Arginase</td>
<td>Erythrocytes</td>
<td>+ (Sequencing)</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td></td>
</tr>
</tbody>
</table>

References

- Summar M & Tuchman M. Proceedings of a Consensus Conference for the management of patients with Urea Cycle Disorders. J Pediatr 2001; 138: S1-S80
- Champion M. Hyperammonaemia. British Inherited Metabolic Disease Group Newsletter 2001; 20: 16-21
- Sudden Unexpected death in Infancy - the report of a working group Royal College of Pathologists and Royal College of Paediatrics and Child Health September 2004 p29
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