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Guidelines

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:

Premature neonate	**<150 µmol/L**
Term neonate	**<100 µmol/L**
Infant & child	**<40 µmol/L**

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. (see Appendix –Measurement of Ammonia in Blood/Plasma)
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 hyperammmonaemia** can occur due to a variety of other causes including hepatic and/or other organ dysfunction.

When to suspect hyperammonaemia? / Clinical Presentation

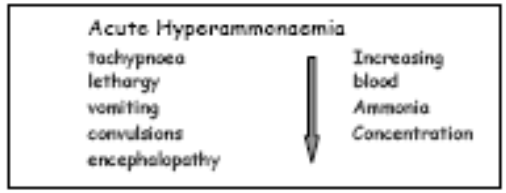
Ammonia is **neurotoxic**; therefore the principal clinical features are neurological.
There is a spectrum of clinical presentation which ranges from an acutely presenting, catastrophic illness in the newborn period to a more insidious, less severe and episodic clinical course in older infants, children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

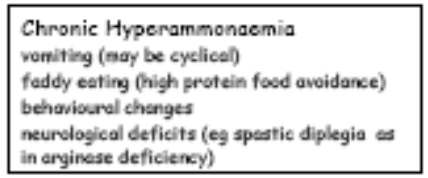


Later infancy, childhood and adulthood

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.

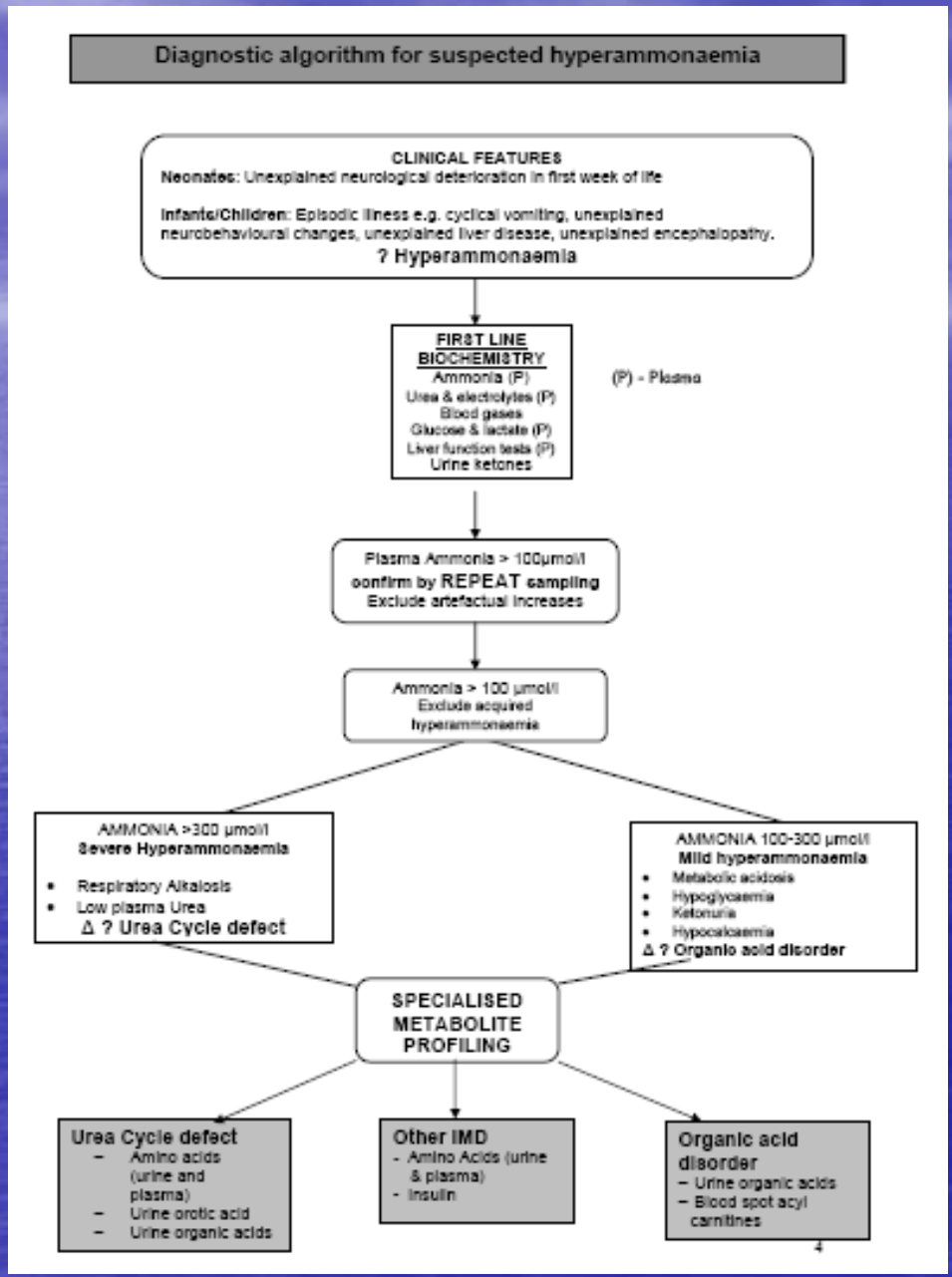
The presentation can also be fluctuating and episodic with mild neurological manifestations such as behavioural disturbances, headaches and vomiting or more severe coma and convulsions.

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.



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 > 100µmol/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

Interpretation of hyperammonaemia and first line investigations

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.

Plasma Ammonia (µmol/L)	Interpretation
<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)

Enzyme defects of the urea cycle:	N-acetyl glutamate synthetase (NAGS)
	Carbamyl phosphate synthetase
	Ornithine transcarbamylase (OTC) – most common (X-linked disorder)
	Argininosuccinate synthetase
	Argininosuccinate lyase
	Arginase
Defects in organic acid metabolism:	
	Propionic acidaemia
	Methylmalonic acidaemia
	Isovaleric acidaemia
	Hydroxymethylglutaryl CoA lyase deficiency
	Fatty acid oxidation defects
Other IMD:	Hyperornithinaemia, hyperammonaemia, homocitrullinuria syndrome (HHH)
	Lysinuric protein intolerance
	Hyperinsulinism hyperammonaemia syndrome

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

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

Table 3:- First line biochemical investigations to investigate hyperammonaemia

Test	Comments
Urea (plasma)	May be inappropriately low compared to other measures of dehydration/renal function (cf creatinine) in urea cycle disorders.
Blood gases	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.
Liver function tests (plasma)	Usually normal in urea cycle disorders but there may be mild elevations in liver enzymes.
Sodium, potassium (plasma)	Not usually abnormal in urea cycle disorders.
Calcium (plasma)	Hypocalcaemia is a feature of organic acid disorders.
Lactate (plasma/blood)	May be non - specifically raised in urea cycle disorders. (see Metbionet guidelines for investigation of Lactic Acidosis)
Glucose (plasma/blood)	Hypoglycaemia is NOT a feature of urea cycle defects (see Metbionet guidelines for investigation of hypoglycaemia)
Urine ketones	Increased in disorders of organic acid metabolism.

Table 4:- Specialised Metabolic Investigations

Test	Interpretation
Amino acids (plasma)	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 defieicncy. **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

(Consult the Network Metabolic Assay Directory www.metbio.net and Genetics Testing Network Directory www.ukgtn.org for information on test availability).

Enzyme Deficency	Enzymology	Molecular Tests
N- acetyl glutamate synthetase	Liver	+
Carbamyl phosphate synthetase	Liver	+ (linkage)
Ornithine transcarbamylase	Liver	+
Arginosuccinic acid synthetase	Cultured fibroblasts	+
Arginosuccinic acid lyase	Erythrocytes Cultured fibroblasts	+ (Sequencing)
Arginase	Erythrocytes Liver	+ (Sequencing)

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Guidelines

- Guidelines for the Investigation of Hyperammonaemia for Inherited Metabolic Disorders
- Guidelines for the Biochemical Investigation of Patients with Foetal and Neonatal Hydrops
- Guidelines for Investigation of Fits and Seizures (Instruction Sheet for CSF sample collection)
- Guidelines for the Investigation of Hypoglycaemia in Infants and Children
- Appendix - Notes on the measurement of ammonia in blood/plasma
- Skin Biopsy - Information Sheet for parents/carers
- Skin Biopsy - Consent form
- Neonatal Jaundice in Inherited Metabolic Disorders