Urea cycle disorders

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Most patients with urea cycle disorders who present as neonates, do so with deteriorating feeding, drowsiness and tachypnoea, following a short initial period when they appear well. The plasma ammonia should be measured at the same time as the septic screen in such patients. Ammonia levels above 200 μmol/l are usually caused by inherited metabolic diseases and it is essential to make a diagnosis for genetic counselling, even if the patients die. The aim of treatment is to lower the ammonia concentrations as fast as possible. Sodium benzoate, sodium phenylbutyrate and arginine can exploit alternative pathways for the elimination of nitrogen but haemodialysis or haemofiltration should be instituted if ammonia concentrations are >500 μmol/l or if they do not fall promptly. Long-term management involves drugs, dietary protein restriction and use of an emergency regimen during illness. Severe hyperammonaemia is usually associated with irreversible neurological damage, particularly if levels have been above 800 μmol/l for >24 hours, and the option of withdrawing treatment should be discussed with the family.

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intensive care. The babies then often develop a wide range of secondary complications such as disordered liver function that obscures the primary condition. Untreated, most babies will die, often with complications such as cerebral or pulmonary haemorrhage. Some survive but they are invariably handicapped, usually severely.

Patients with arginase deficiency usually present after the neonatal period with spasticity in the legs and developmental delay but seldom have symptomatic hyperammonaemia. On the other hand, neonatal hyperammonaemia is well recognized in patients with defects of the mitochondrial ornithine transporter, an essential component of the urea cycle (Hyperornithinaemia, Hyperammonaemia, Homocitrullinuria syndrome). Severe neonatal hyperammonaemia also occurs in patients with ornithine aminotransferase deficiency [1,2], a defect that more commonly presents in adults with cataracts and gyrate atrophy of the choroid and retina.

Differential diagnosis

The differential diagnosis of hyperammonaemia is wide and is summarized in Table 1. The most common differential diagnoses of severe hyperammonaemia are organic acidemias, particularly propionic and methylmalonic acidemia. It is important to recognize that patients with these disorders may have marked hyperammonaemia with a respiratory alkalosis without acidosis or ketosis. Transient hyperammonaemia of the newborn (THAN) is an ill-understood condition, possibly related to immaturity of liver metabolism or hepatic vascular disease. Plasma ammonia levels may be very high initially but no underlying metabolic disease is found. Although babies with THAN are often born prematurely with early onset of symptoms [3], it may be difficult to distinguish between urea cycle disorders and this disorder on clinical grounds. The incidence of THAN appears to have been falling over recent years in many centres around the world. Less severe hyperammonaemia is common, both in other metabolic disorders and acquired illness such as sepsis and perinatal asphyxia. Babies with systemic herpes simplex, particularly involving the liver, may have marked hyperammonaemia without obvious signs.

Investigations

Routine tests are not helpful for establishing the diagnosis of hyperammonaemia. The most important diagnostic test in urea cycle disorders is measurement of the plasma ammonia concentration. In healthy neonates plasma ammonia is normally less than 65 \(\mu\text{mol/l}\) [4], but may be raised as a result of a high protein intake, difficult venepuncture or a haemolysed blood sample. In sick neonates (for example, those with sepsis or perinatal asphyxia), plasma ammonia concentrations may increase up to 180 \(\mu\text{mol/l}\). Patients with inborn errors presenting in the newborn period usually have concentrations greater than 200 \(\mu\text{mol/l}\), often very much greater.

Ammonia levels can rise rapidly in patients with urea cycle disorders. Thus, plasma ammonia measurement should be repeated after a few hours, even if it is only modestly elevated.

In cases of significant hyperammonaemia, the following investigations should be performed immediately:

- Blood pH and gases
- Plasma urea and creatinine, electrolytes, glucose

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<th>Table 1. Differential diagnosis of hyperammonaemia</th>
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<td><strong>Acquired disorders</strong></td>
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<td>Herpes simplex – systemic infection</td>
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<td>Liver failure (rare in neonates)</td>
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<td>Infection with urease positive bacteria (if urinary tract stasis)</td>
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Liver function tests and clotting studies

Plasma amino acids

Urine organic acids, orotic acid and amino acids

Plasma free and acylcarnitines

The plasma amino acids and urine organic acids are very urgent.

In all urea cycle disorders there is accumulation of glutamine and alanine. There are also increased concentrations of the amino acids immediately proximal to the block in the metabolic pathway and decreased concentrations of those beyond the block (Fig. 1). Thus, in citrullinaemia, argininosuccinic aciduria (ASA) and arginase deficiency, the plasma amino acids are usually diagnostic (Table 2).

Orotic acid and orotidine are excreted in excess in the urine if there is a metabolic block distal to the formation of carbamoyl phosphate. In these disorders carbamoyl phosphate accumulates, leaves the mitochondrion and enters the pathway for the de novo synthesis of pyrimidines in the cytosol (Fig. 1). Measurement of urinary orotic acid is particularly helpful for distinguishing ornithine transcarbamoylase (OTC) deficiency from carbamoyl phosphate synthetase (CPS) or N-acetyl glutamate synthetase (NAGS) deficiencies.

Diagnoses can generally be confirmed by measuring the enzyme activity in an appropriate tissue (Table 2). This is the only way to distinguish between CPS and NAGS deficiencies. Assays of CPS are well-established but measurement of NAGS activity is not straightforward. Patients with NAGS deficiency generally show a clinical response to N-carbamyl glutamate, an orally active analogue of N-acetyl glutamate, but this is unreliable for diagnosis because a response is also seen in some patients with CPS deficiency [5].

Other investigations will detect complications. In the late stages of hyperammonaemia patients may have marked disturbances of liver function with disordered clotting, renal failure and hypocalcaemia. In the later stages of hyperammonaemic encephalopathy, brain imaging may show cerebral oedema or intracranial haemorrhage.

If the patient seems likely to die it is essential to collect the appropriate specimens, since otherwise the diagnosis cannot be confirmed:

- Plasma (heparinized, separated and deep frozen)
- Blood spots on filter paper for acylcarnitines
- Urine (deep frozen in a plain tube)
- Blood for DNA (anticoagulated with EDTA and deep frozen)
- Skin for fibroblast culture taken with sterile precautions into medium and stored at 4–8°C, not frozen
- Liver, snap frozen for enzymology.

<table>
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<th>Table 2. Diagnostic tests in urea cycle defects</th>
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<td>Disorder</td>
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AR: autosomal recessive; RBC: red blood cells; N: normal.
Pathogenic mechanisms

Ammonia induces many electrophysiological, vascular and biochemical changes in experimental systems but it is not known to what extent these are relevant to the problems of hyperammonaemia in man [6]. Ammonia increases the transport of tryptophan across the blood brain barrier, which then leads to an increased production and release of serotonin [7]. Some of the symptoms of hyperammonaemia can be explained on this basis and the dietary tryptophan restriction has reversed anorexia in some patients with urea cycle disorders [8].

Glutamine has been shown to accumulate in the brain during hyperammonaemia in experimental animals and in man in vivo, using proton nuclear magnetic resonance spectroscopy [9]. The concentrations are such that the increase in osmolality could be responsible for cellular swelling and cerebral oedema.

Acute management

In the neonatal period the immediate goal of treatment is to control the metabolic derangement. Once plasma ammonia concentrations are greater than 500 μmol/l this is urgent. The strategies used are to stop any dietary protein and give a high energy intake, reduce plasma ammonia concentrations with dialysis and utilize alternative pathways of nitrogen excretion. The emergency management of neonatal hyperammonaemia is summarized in Table 3.

Protein and energy intake

As soon as hyperammonaemia is suspected all intake of protein should be stopped and a high energy intake given, either orally or intravenously.

Table 3. The emergency treatment of neonatal hyperammonaemia

- **General neonatal supportive care** e.g. ventilation (particularly prior to transfer) treatment of sepsis, seizures etc.
- **Stop protein intake**
- **Give a high energy intake**
  - either (a) oral
    - (i) 10% soluble glucose polymer (higher concentrations may be given if they are tolerated)
    - (ii) protein free formula (80056 (Mead Johnson); Duocal (SHS Ltd))
  - or (b) intravenously
    - (i) 10% glucose by peripheral infusion
    - (ii) 10–25% glucose by central venous line

Fluid volumes may be restricted if there is concern about cerebral oedema

- **Alternative pathways for nitrogen excretion**
  - Sodium benzoate up to 500 mg/kg/day – oral or intravenously
  - Sodium phenylbutyrate up to 600 mg/kg/day
  - L-arginine
    - In citrullinaemia and ASA – up to 700 mg/kg/day
    - In OTC deficiency and CPS deficiency – up to 150 mg/kg/day
  - L-citrulline
    - In OTC deficiency and CPS deficiency up to 170 mg/kg/day instead of arginine

For the emergency treatment of hyperammonaemia before the diagnosis is known, some centres consider the following to be a safer alternative:

- L-arginine 300 mg/kg/day
- L-carnitine 200 mg/kg/day

Both can be given orally or intravenously

- **Dialysis** (haemodialysis, haemodiafiltration or haemofiltration)
  - Start immediately if plasma ammonia >500 μmol/l or if ammonia does not fall with the above measures.

Note these regimens are not nutritionally complete and will cause malnutrition. They must not be continued longer than absolutely necessary.


**Dialysis**

Once the plasma ammonia concentrations are greater than 500 μmol/l, it is essential to take steps to reduce this as quickly as possible. Haemodialysis or haemofiltration should be used rather than peritoneal dialysis, which is much less effective. The exact management will depend on the facilities and experience available. The easiest and most widely used is continuous veno-veno-haemofiltration. Although there may be theoretical concerns about rapid removal of ammonia and other metabolites, there are no reports of complications from rapid fluid shifts or by other mechanisms.

**Alternative pathways for nitrogen excretion**

A major advance in this field has been the development of compounds that are conjugated to amino acids and rapidly excreted \[10,11\]. The effect of the administration of these substances is that nitrogen is excreted as compounds other than urea and hence the load on the urea cycle is reduced (Fig. 1). The first compound introduced was sodium benzoate. Benzoate is conjugated with glycine to form hippurate, which is rapidly excreted. For each mol of benzoate given, 1 mol of nitrogen is removed. The major side effects are nausea, vomiting and irritability. In the newborn, conjugation may require enzyme induction – hence, conjugation may be incomplete at the very time when it is needed most (C. Bachmann, personal communication). There is also an increased risk of toxicity. Theoretically, sodium benzoate might precipitate kernicterus and it is particularly important to take into account the high sodium content in neonates. The next drug used was phenylacetate but this has now been superseded by phenylbutyrate, because the former has a peculiarly unpleasant clinging mousy odour. Phenylbutyrate is oxidized in the liver to phenylacetate, which is then conjugated with glutamine. The resulting phenylacetyl-glutamine is excreted in the urine and hence 2 mol of nitrogen are lost for each mol of phenylbutyrate given. Accidental overdoses of sodium benzoate and sodium phenylbutyrate have caused metabolic acidosis, cerebral oedema and circulatory collapse \[12\].

In patients with citrullinaemia and ASA, nitrogen can be excreted in the form of citrulline and argininosuccinic acid, respectively. The formation of these metabolites is limited by the low ornithine levels that result from the metabolic block (Fig. 1). Arginine supplements can replenish the supply of ornithine, maximizing the excretion of citrulline and argininosuccinic acid. Arginine doses of up to 700 mg/kg/day may be used. Though the concentrations of citrulline or argininosuccinate rise, these compounds are thought to have less adverse effects than the accumulation of ammonia and glutamine.

Alternative treatment regimens have been proposed because of concerns about the potential toxicity of sodium benzoate and sodium phenylbutyrate. Some authorities, for example, advocate giving only arginine and carnitine before the diagnosis is known, if the ammonia concentration does not warrant dialysis (Table 3). No studies have been done comparing these different regimens.

**Long-term treatment**

Once the acute illness has been controlled, it is necessary to reintroduce an oral feed containing protein and energy. The aim of long-term treatment is to correct the biochemical disorder and yet ensure that all the nutritional needs are met. For severely affected patients this can be difficult. The major strategies used are to give a low protein diet, to utilize alternative pathways of nitrogen excretion and to replace nutrients that are deficient.

**Low protein diet**

All patients with urea cycle disorders presenting in the newborn period require a strict low protein diet. The protein tolerance of patients varies considerably and depends on factors such as age and growth rate as well as the residual enzyme activity. When protein is first introduced there is often a rise in the plasma ammonia concentration and it is necessary to persist with the feeds to get the baby anabolic. Once this is achieved, metabolic control during early infancy is often straightforward and the patients may need 1.8–2 g/kg/day of protein or sometimes even more during very rapid growth. All diets must, of course, be nutritionally complete and meet the requirements of growth and normal development.

**Essential amino acids**

In the most severe variants it may not be possible to achieve good metabolic control and satisfactory
nutrition with restriction of natural protein alone. In these patients some of the natural protein may be replaced with an essential amino acid mixture, giving up to 0.7 g/kg/d. Essential amino acid mixtures ensure that there are adequate precursors for protein synthesis whilst minimizing the nitrogen load to be excreted.

**Arginine and citrulline**

Arginine is normally a non-essential amino acid because it is synthesized within the urea cycle. For this reason, all patients with urea cycle disorders except those with arginase deficiency are likely to need a supplement of arginine to replace that which is not synthesized [13]. The aim should be to maintain plasma arginine concentrations between 50 and 200 μmol/l. In citrullinaemia and ASA, patients will need up to 500 mg/kg/day. For most patients with OTC and CPS deficiencies, a dose of 100–150 mg/kg/day appears to be sufficient. Severely affected patients with these disorders may profit from using citrulline (up to 170 mg/kg/day) instead of arginine as this will utilize an additional molecule of nitrogen.

**Alternative pathways for nitrogen excretion**

Patients continue to need alternative pathway therapy to maintain good metabolic control, although full doses may not be necessary during the phases of rapid growth. For each patient there is a balance between the protein intake and the dose of their medicines to achieve good metabolic control. If patients can take large doses of sodium benzoate and sodium phenylbutyrate, it will increase their protein tolerance but if they only manage small doses, their diet will have to be stricter.

**Other medication**

N-carbamyl glutamate can be used in NAGS deficiency to replace the missing compound, as it is active orally. The dose is 100 mg/kg/day [14]. Patients who respond may require treatment only with this compound.

Anticonvulsants may be needed for patients with urea cycle disorders but sodium valproate should not be used as this drug may precipitate fatal decompensation particularly in OTC deficient patients [15].

**Monitoring**

All treatment must be monitored with regular estimations of plasma ammonia and quantitative amino acids, paying particular attention to the concentration of glutamine and essential amino acids. The aim is to keep plasma ammonia less than 80 μmol/l and plasma glutamine less than 800 μmol/l [16], but in practice 1000 μmol/l is probably more realistic, together with concentrations of essential amino acids within the normal range.

**Management of acute illness**

All patients with urea cycle disorders are at risk of acute decompensation with acute hyperammonaemia. This can be precipitated by metabolic stresses, such as fasting, a large protein load, infection, anaesthesia and surgery but in patients with severe variants there may be no very obvious reason. All patients should have detailed instructions of what to do when they are at risk. We routinely use a three-stage procedure. If the patient is off colour, the protein is reduced and more carbohydrate given. If symptoms continue, protein should be stopped and a high energy intake given together with their medication both day and night. If they refuse or vomit their emergency drinks or medicines, or show any signs of encephalopathy, they should go to hospital urgently for assessment and intravenous therapy. For further practical details see [17].

**Prognosis**

The prognosis in these disorders is closely related to the age of the patient and their condition at the time of diagnosis. For those patients who present with symptomatic hyperammonaemia in the newborn period, the outlook is very poor. Even with the most aggressive treatment, the majority of the survivors will be handicapped. Those who are treated prospectively do much better but there may still be significant complications [18]. For these patients there remains a serious risk of
decompensation and careful consideration should be given to early liver transplantation, which may offer the hope of a better long-term outlook [19,20].

Genetics and prenatal diagnosis

The genes for all the urea cycle enzymes except N-acetyl glutamate synthetase have been mapped, isolated and fully characterized. Many mutations have been described. The commonest urea cycle disorder is OTC deficiency. This is an X-linked disorder in which molecular genetic studies are particularly helpful. When the diagnosis of OTC deficiency is established, a careful family history should be taken and the mother’s carrier status should be assessed. If the mutation is unknown, the most convenient investigation is currently the allopurinol test. This detects increased synthesis of orotic acid and orotidine; allopurinol inhibits the metabolism of these pyrimidines, enhancing their excretion and allowing the detection of even asymptomatic carriers (Fig. 1) [21,22]. The allopurinol test is easier than protein or alanine loading tests and carries no risk of hyperammonaemia. Prenatal diagnosis is possible in most families using informative polymorphisms if the mutation itself has not been identified. Whilst the phenotype of the males can be predicted, that of the females cannot because of the random inactivation of the X chromosome. This presents a problem when counselling families, but the prognosis for females who are treated prospectively from birth is good.

All the other urea cycle disorders have autosomal recessive inheritance and prenatal diagnosis is possible for all except NAGS deficiency. For CPS deficiency, prenatal diagnosis using closely linked gene markers is now possible in a substantial proportion of families. If the molecular genetic studies are uninformative, prenatal liver biopsy is an alternative. Citrullinaemia and ASA can both be diagnosed on chorionic villus biopsy. Arginase deficiency can be diagnosed either by molecular genetic studies or, if they are not informative, on a fetal blood sample.

Conclusions

It is important to have a low threshold for measuring the plasma ammonia in neonates. Severe hyperammonaemia is a neonatal emergency with a high risk of neurological damage. Unless the parents wish treatment to be withdrawn, ammonia levels should be lowered as fast as possible, usually by haemofiltration or dialysis. Most causes of significant hyperammonaemia are genetic and it is important to make a diagnosis even if the patient dies.

References


