ABSTRACT
Hyperammonaemia (HA) as a consequence of numerous primary or secondary causes, gives rise to clinical manifestations due to its toxic effects on the brain. The neurological consequences broadly reflect the ammonia level, duration and age, with paediatric patients being more susceptible. Drug-induced HA may arise due to either decreased ammonia elimination or increased production. This is associated most frequently with use of valproate and presents a dilemma between ongoing therapeutic need, toxicity and the possibility of an alternative cause. As there is no specific test for drug-induced HA, prompt discussion with a metabolic physician is recommended, as the neurotoxic effects are time-dependent. Specific guidelines for managing drug-induced HA have yet to be published and hence the treatment approach outlined in this review reflects that outlined in relevant urea cycle disorder guidelines.

INTRODUCTION
Hyperammonaemia (HA) (>40 μmol/L, 68 μg/L in adults, although method dependent) arises due to a number of causes and in general, clinical manifestations broadly reflect the degree of ammonia elevation, arising due to central nervous system toxicity.7 Ammonia is a highly potent neurotoxin and severe acute HA is a medical emergency. It may result in a rapidly progressive, often fatal, encephalopathy with brain damage. Chronic HA may cause progressive cognitive impairment, behavioural abnormalities and neuropsychiatric illness. Patients may present with encephalopathy, with confusion, agitation, ataxia, seizures or coma, particularly as values of plasma ammonia increase above 100–200 μmol/L, however the threshold for clinical symptoms is highly variable.5 Clinical features are non-specific and can arise in more common conditions such as sepsis. As a consequence of this, HA is often missed as it is not part of the standard panel for encephalopathy in adult general medicine outside of hepatology. In adults, most cases arise due to liver failure, and although rare there is a growing appreciation that inherited metabolic disorders (IMDs) can present at any age.

An important source of adult-onset non-cirrhotic HA are IMDs which lead to primary or secondary urea cycle disorders (UCDs) and conditions associated with increased ammonia production such as urea-producing infections4 (see tables 1 and 2). Although the overall estimated incidence for all UCDs is rare at 1 in 35 000, there are some cohort data supporting the view that 50% present in adolescence/adulthood and of those presenting in adulthood 50% present acutely.5–7 Timely management of the first presentation is an important predictor of neurological outcome with persistent values >360 μmol/L in early onset and >200 μmol/L in adult onset, being associated with poorer neurological outcomes.8–10 This is important and reinforces the need to consider checking ammonia in any patient of any age with encephalopathy, as early treatment can reverse the neurological deterioration.

This review provides a brief overview of the biochemistry of primary and secondary causes of HA and reviews in detail drug-induced HA and its management in adults. Much has been written about the preanalytical factors that can affect ammonia analysis and we assess this in a systematic fashion. A similar approach is used in describing relevant diagnostic testing and management, to avoid missing relevant primary disorders and treatment delays that may exacerbate neurological outcomes.

PATHOPHYSIOLOGY
Ammonia is a metabolic by-product of all cells. The major contributors to the body load are the gastrointestinal (GI) tract, skeletal muscle and kidney. Ammonia is produced in the GI tract from three sources: bacterial urease breakdown of circulating urea, bacterial deamination of luminal protein and glutaminase breakdown of glutamine from circulating glutamine.9 Large amounts of ammonia are generated in skeletal muscle, especially during activity/seizures mainly from deamidation of AMP, and to a lesser extent from amino acid catabolism. In kidneys it is released from glutamine entering the proximal tubular cells from the glomerular filtrate and the circulation.

Because of its neurotoxicity, arterial blood concentration is normally tightly regulated and does not vary much even after meals with the main sites of production being the GI tract, skeletal muscle and the kidney. At physiological pH in body fluids, ammonia (NH₃) is predominantly hydrogenated to ammonium (NH₄⁺). At neutral pH NH₄⁺ predominates, which is useful as this limits its perfusion across cell membranes. Throughout the text ‘ammonia’ refers to summated NH₃ and NH₄⁺ concentrations, which is the common convention.

Ammonia is toxic to the brain but not to other tissues and readily crosses the blood-brain barrier.16 Average levels of brain ammonia are normally approximately twice those of blood. In experimental animals with acute liver failure, brain ammonia flux may be up to 45-fold higher than normal.13 A number of theories have been proposed to explain HA-induced brain damage.12–18 It has been postulated that increased intracerebral ammonia levels may interfere with mitochondrial function, may disrupt inhibitory and excitatory neurotransmission, and lead to excessive glutamatergic glial accumulation leading to astrocyte swelling. One of the
CPS1 (see table NAG acts as an activator of carbamoylphosphate synthetase mitochondrial, three cytosolic), together with N-organ that houses the complete urea cycle of five enzymes (two of ammonia into urea requires the function of the six enzymes the liver, which is then excreted by the kidneys. The conversion body. This is achieved by conversion to urea via the urea cycle in for biosynthetic processes must be excreted rapidly from the

**Table 1** Inherited metabolic disorders which may cause primary hyperammonaemia (HA)\(^8\)

<table>
<thead>
<tr>
<th>Urea cycle disorders</th>
<th>Enzyme Deficiency</th>
<th>Transporter Defect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytoplasmic ornithine carbamoyl transferase deficiency</td>
<td>Ornithine carbamoyltransferase deficiency</td>
<td>1. Citrin deficiency-aspartate glutamate carrier</td>
</tr>
<tr>
<td>Citrullinemia</td>
<td>Ornithine carbamoyltransferase deficiency</td>
<td>2. Hyperornithinaemia, HA and homocitrullinuria (3 hour syndrome)-ornithine transport</td>
</tr>
<tr>
<td>Argininosuccinic aciduria</td>
<td>Ornithine carbamoyltransferase deficiency</td>
<td></td>
</tr>
<tr>
<td>Argininaemia</td>
<td>Ornithine carbamoyltransferase deficiency</td>
<td></td>
</tr>
<tr>
<td>N-Acetylglutamate synthetase deficiency</td>
<td>Ornithine carbamoyltransferase deficiency</td>
<td></td>
</tr>
</tbody>
</table>

most concerning outcomes of this pathology is that severe HA can cause cerebral oedema with a rise in intracranial pressure that can lead to herniation and brainstem compression. Large amounts of NH\(_3\) are produced daily. NH\(_3\), not recyled for biosynthetic processes must be excreted rapidly from the body. This is achieved by conversion to urea via the urea cycle in the liver, which is then excreted by the kidneys. The conversion of ammonia into urea requires the function of the six enzymes and two transporters of the urea cycle. The liver is the only organ that houses the complete urea cycle of five enzymes (two mitochondrial, three cytosolic), together with N-acetylglutamate synthetase (NAGS) which produces N-acetylglutamate (NAG). NAG acts as an activator of carbamoylphosphate synthetase (CPS1) (see table 1 and figure 1).

In order to transfer NH\(_3\), safely from the tissues to the liver via the circulation, it is combined with glutamate by glutamine synthetase to produce non-toxic glutamine. Some is extracted by the kidneys and the immune system for biosynthesis. Most is taken up by the small intestinal mucosa. Here NH\(_3\), is released by glutaminase and transported directly to the liver in the portal circulation.

Cytosolic ornithine is transported into the mitochondria in exchange for intramitochondrial citrulline by the ornithine/citrulline carrier encoded by the SLC25A15 gene. Citrin, a liver transporter encoded by the SLC25A13 gene, exports aspartate from the mitochondria in exchange for glutamate that is used in the formation of NAG (figure 1). Two atoms of nitrogen are converted to urea for each turn of the urea cycle. One comes from ammonia and the other comes via the citrin carrier and the amino acid pool in the form of aspartate. The latter derives from the amino group of alanine, which is transferred to oxaloacetate to produce aspartate. NAGS produces NAG which is an essential cofactor for CPS1, the first and rate-limiting enzyme of the urea cycle.\(^{19,20}\)

**Table 2** Differential diagnosis and mechanism of primary and secondary hyperammonaemia (HA)\(^{11,79,81}\)

<table>
<thead>
<tr>
<th>Increased ammonia production</th>
<th>Condition</th>
<th>Suggested mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise/trauma</td>
<td>AMP deamination</td>
<td></td>
</tr>
<tr>
<td>Gastric bypass/starvation</td>
<td>Increased protein catabolism</td>
<td></td>
</tr>
<tr>
<td>GI haemorrhage</td>
<td>Excess protein/nitrogen load</td>
<td></td>
</tr>
<tr>
<td>Total parenteral nutrition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infections with urease-producing bacteria</td>
<td>Urinary tract infection, with relevant organisms</td>
<td></td>
</tr>
<tr>
<td>Decreased ammonia elimination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute or chronic liver disease</td>
<td>Reduced urea cycle, glutamine synthesis; portosystemic shunt</td>
<td></td>
</tr>
<tr>
<td>Urea cycle disorder</td>
<td>Enzyme block or substrate transport affecting urea cycle</td>
<td></td>
</tr>
<tr>
<td>Fatty acid disorder of oxidation</td>
<td>Lack of acetyl-CoA leading to reduced CPS1 activity</td>
<td></td>
</tr>
<tr>
<td>Organic acidemia</td>
<td>NAGS inhibition by relevant increased acid</td>
<td></td>
</tr>
<tr>
<td>Carbonic anhydrase Va deficiency</td>
<td>Lack of bicarb leading to reduced CPS1</td>
<td></td>
</tr>
<tr>
<td>Mitochondrial disorders</td>
<td>Impaired ATP production/substrate</td>
<td></td>
</tr>
<tr>
<td>Ornithine aminotransferase deficiency</td>
<td>Lack of ornithine affecting OTC, urea cycle defects</td>
<td></td>
</tr>
<tr>
<td>Glutamine synthase deficiency</td>
<td>Decreased glutamine and hence ammonia clearance</td>
<td></td>
</tr>
<tr>
<td>Lysinuric protein intolerance</td>
<td>Lack of urea cycle ornithine and arginine</td>
<td></td>
</tr>
<tr>
<td>CPS1, carbamoylphosphate synthetase; GI, gastrointestinal; NAGS, N-acetylglutamate synthetase; OTC, ornithine transcarbamylase</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Drug-induced HA**

A number of medications have been associated with HA, with valproate being the most common. Confounding factors such as sepsis and liver disease, and lack of clear mechanisms for some drugs weaken the evidence for association. In a search of the WHO adverse drug reaction database (Vigibase), for the period 1967 to 8 May 2019, 73 drugs were reported to be suspected of causing HA. table 3 lists those most frequently recorded; 63% were associated with valproate, 11% with fluourouracil, 5% topiramate and the rest accounting less than 5% each of overall cases.\(^{21}\) Vigibase is a retrospective observational database used to record all adverse drug reactions from over 130 countries, and the table below lists the most frequently recorded.

The following were excluded: drugs reported less than three times and drugs used to treat HA or hepatic encephalopathy. There are some obvious limitations to using this as a source as it relies on spontaneous notification, retrospectively, so delay may affect recall, incomplete information, lack of direct inspection of laboratory data and undernotification. For instance, the case numbers with liver failure are low compared with the estimated population prevalence and this may represent the fact that clinicians attributed the HA (in those cases excluded) to liver disease rather than to a particular drug.

Part of the challenge in dealing with such HA is teasing out confounders such as liver disease, sepsis, malnutrition, IMD or a drug effect. Although for the drugs we have tabulated in table 3 there is some mechanistic linking the drug to HA, for some medications this is not the case. In addition, this is hampered by the absence of a specific test to rule in or rule out the drug-associated HA. To strengthen the evidence for association, Weiss et al extended their Vigibase study by checking potential causation checking time of onset of HA to discontinuation and whether this correlated with the product characteristics documented from the European Medicines Agency and the Federal Drug Authority.\(^{21,22}\) Of interest is the fact that 74% of the cases were reported in the last decade of the search period; a median time of onset of HA following drug commencement was 13 days (IQR 2–59 days).\(^{23}\) Of the 73 drugs, 10 described the association with HA in the drug product characteristics (valproic acid (VPA), valproamide (VPA prodrug), topiramate, asparaginase, fluorouracil, haloperidol, pegaspargase, zonisamide, deferasirox, amphotericin B). table 3 lists the most frequently recorded drugs...
associated with HA. The frequency of HA was uncommon for asparaginase (between 1/100 and 1/1000 cases), rare (between 1/1000 and 1/10000) for VPA, valpromide, topiramate and undetermined for the other six drugs. Of the remaining 61 drugs, 6 were published in reference databases/books, 45 were described in case reports/series and 10 had never been published. This might reflect greater awareness of HA as a drug complication and/or a higher incidence with introduction of new drugs (see reference22 for a comprehensive list).

**VALPROATE**

VPA (2-n-propylpentanoic acid) is a C8-branch chain fatty acid that has been used for many years to treat epilepsy, more recently as mood stabiliser for a number of psychiatric conditions and migraine. In a recent audit of general hospitalised adults who received VPA, 20.4% developed HA.23 This audit included those patients admitted over a 1-year period in one hospital, age >18 years, receiving at least one dose of VPA. Patients with cirrhosis were excluded from the audit. HA was defined as above the institutional reference range of >23.6 µmol/L, 162 patients were included and ammonia ranged from 12 µmol/L to 75 µmol/L. Epilepsy and psychiatric condition were the most frequent indication for VPA and the mean plasma ammonia in symptomatic patients was 39.5 µmol/L. A limit for this paper is that it was retrospective, duration of VPA use was not recorded and neither was dose. However, the strengths of the study include the large sample size and general hospital location, rather than a specific medical discipline.

A similar study conducted on patients admitted to a psychiatric medicine unit had a prevalence of HA of 36%.24 Symptoms of VPA-induced HA can vary from asymptomatic HA to VPA-induced hyperammonaemic life-threatening encephalopathy.25 Various mechanisms have been implicated in the development of VPA-induced HA, mainly via effects on the liver and less so on the kidney. VPA β-oxidation leads to valproyl-CoA (valproyl Coenzyme A) which directly inhibits NAGS and induces secondary carnitine deficiency which leads to decreased acetyl-CoA that may also contribute to VPA-induced HA.26 27 (figure 1). Hepatotoxicity (non-specific variable) may accompany this and a dose-dependent effect on plasma ammonia has been observed with increasing VPA doses and during use with other antiepileptic drugs (AEDs). Elevations in plasma ammonia levels, as high as 140 µmol/L, were well tolerated, and VPA dose reductions were not necessary.28 However, in an audit of AED use, in the VPA only group, ammonia concentration ranges from 60 µmol/L to 400 µmol/L were recorded, with some being well above the treatment threshold (see below for treatment section).29 Risk factors for hepatotoxicity associated with VPA include young age, polytherapy and developmental delay.30 Although rare, coexisting UCD suggested by a sudden

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**Table 3** List of drugs commonly associated with HA

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cases of HA</th>
<th>Serious events (%)</th>
<th>Fatal events (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Valproate</td>
<td>1260</td>
<td>768 (61)</td>
<td>54 (4)</td>
</tr>
<tr>
<td>2 Fluorouracil</td>
<td>221</td>
<td>213 (96)</td>
<td>20 (9)</td>
</tr>
<tr>
<td>3 Topiramate</td>
<td>96</td>
<td>65 (68)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>4 Oxaliplatin</td>
<td>74</td>
<td>68 (92)</td>
<td>12 (16)</td>
</tr>
<tr>
<td>5 Asparaginase</td>
<td>71</td>
<td>50 (70)</td>
<td>6 (8)</td>
</tr>
</tbody>
</table>

Columns 3 lists the absolute number of cases associated with HA for each drug. Columns 4 and 5 list the absolute number and % in brackets of serious and fatal events. For serious events these were defined as severe case, death, life-threatening, caused or prolonged hospitalisation, disabling/incapacitating, congenital anomaly/birth defect, other medically important condition. For seriousness criteria it was possible to choose more than one.

HA, hyperammonaemia.
development of hyperammonaemic crises should be considered. Hyperammonaemic crises maybe triggered by catabolic events, protein overload, infection, GI bleeding or certain drugs.31 32 This needs to be considered when requesting relevant laboratory tests (see below laboratory investigations).

**TOPIRAMATE**

Topiramate (TPM) is a carbonic anhydrase inhibitor used to treat epilepsy and alcohol dependence, and prevent migraines. HA is an uncommon side effect of topiramate and is usually related to combined use with valproate and phenobarbital.33 It is believed that the mechanism implicated in HA involves inhibition of carbonic anhydrase leading to reduced bicarbonate and reduction in activity of the cytosolic enzyme, glutamine synthetase. Reduced bicarbonate may decrease activity of CPS1, the rate-limiting enzyme in the urea cycle (figure 1).34 The reduction in glutamine synthetase levels limits the incorporation of ammonia into glutamate, to form glutamine. Both of these mechanisms can lead to HA; however, it should be noted that both of these studies were in animal models and there are no human studies to date. Values of ammonia of up to 77 µmol/L in a single use of topiramate have been recorded and up to 146 µmol/L in combination with other AEDs as noted above.33–35 Addition of VPA to a patient already taking topiramate may increase the risk of HA.36

**FLUOROURACIL**

5-fluorouracil (5-FU) is a common chemotherapy drug used in the treatment of head and neck, GI, and breast cancers. 5-FU is a pyrimidine analogue that acts principally as a thymidylate synthase inhibitor. After entering the cell, 5-FU is converted into several metabolites. The rate-limiting step of the catabolic process is the conversion of 5-FU to the inactive metabolite dihyd-rofluorouracil which is catalysed by the enzyme dihydroyprymidine dehydrogenase (DPD).37 Decreased metabolism by DPD creates a number of potentially toxic metabolites and hence it is a standard practice to screen for DPD variants (associated with decreased metabolism) that increase this risk, in particular of severe neutropaenia.38 To date there has been some suggestion that such variants in association with other factors contribute to HA. Boilère et al found an association with DPD deficiency in 27% and Milano et al found an association with neurotoxicity in 37%, but did not record ammonia values.39 40

HA has been reported after treatment with 5-FU, with early symptoms being non-specific, such as nausea and vomiting progressing to encephalopathy.41 Large doses of 5-FU induce accumulation of fluoroacetate which can inhibit the enzyme aconitase and result in the impediment of citrate isomerisation. This in turn impair Krebs cycle function reducing ATP42 and oxaloacetate.43 Impairment of ATP-dependent urea cycle results in HA. These are two essential factors in the early proximal part of the urea cycle, with oxaloacetate providing the source of aspartate via aspartate transaminase (figure 1).

The reported incidence of encephalopathy has been around for 10% with HA being around 1.0%.40 43 Values of ammonia up to 522 µmol/L have been recorded, which is well above the therapeutic intervention threshold (see the Treatment section below).40 43 However, it should be borne in mind that often these patients may have multiple confounding factors such as sepsis, shock, sarcopenia and hepatic impairment, that need to be considered. The original reports described an acute cerebellar syndrome associated with HA and since then several case studies and cohort studies have been published.44–47

For clinicians treating such patients the challenge is teasing out often what may not be multifactorial encephalopathy, due to the cancer, medications or malnutrition. A paraneoplastic encephalopathy is more likely to be subacute, however 5FU also interferes with the conversion of thiamine-to-thiamine pyrophosphate (its active form) which can present as Wernicke’s encephalopathy. This is compounded by the fact that such a group is nutritionally vulnerable and may already be deficient in thiamine, among other vitamins.48

**OXALIPLATIN**

Oxaliplatin a member of the platinum chemotherapy class, undergoes non-enzymatic conversion to active derivatives which preferentially bind guanine and cytosine bases in DNA leading to cross linking. This arrests DNA synthesis, with effects not being dependent on cell cycle, being particularly efficient on tumours with high cell turnover.49 50 It can be used to treat a variety of metastatic tumours but is commonly in combination with 5-FU to treat colorectal tumours. The drug causes dose-dependent toxicity on the haematopoietic system and dose-limiting effects on the nervous system causing acute or chronic peripheral neuropathy affecting multiple types of nerve fibres.51

HA has been uncommonly described, usually in combination with 5-FU and hepatic dysfunction, therefore may have synergistic effects, on the urea cycle and might increase the risk for HA.52 53 The exact mechanism however remains unclear but may be related to negative effects on the Krebs cycle impacting the proximal urea cycle. Values of ammonia of up to 200 µmol/L have been recorded comfortably above the treatment threshold (see the Treatment section).54 55

**ASPARAGINASE**

Asparaginase has been a long-standing component of induction, consolidation and maintenance therapy for acute lymphoblastic leukaemia.56 57 The principal of this chemotherapy is that neoplastic cells have reduced capacity to synthesise asparagine and hence need this from the blood.

Asparaginase catalyses the formation of aspartic acid and ammonia from asparagine and hence an increase in ammonia is expected as a consequence of its therapeutic effect. In a prospective cohort study, although 7/10 had HA as expected none were symptomatic and although peak levels increased up to seven times upper-normal limit 24 hours after dose, concentrations fell to normal by 2–3 days.58

Symptomatic HA has previously been reported to be uncommon however in a study where ammonia was checked regardless of symptoms, ammonia >100 µmol/L was found in 7 out of 10 patients being even higher in those where PEG-asparaginase was used, due to the prolonged half-life.59 It is clear therefore that ammonia should be checked as a regular part of therapeutic management regardless of symptoms, otherwise this may be missed. Values of ammonia up to 400 µmol/L have been documented in setting of asparaginase use, well above the therapeutic threshold for intervention for HA (see the Treatment section). Other metabolic effects such as hypertriglyceridaemia due to antagonistic effect on lipoprotein lipase have also been described.

**OTHER MEDICATIONS**

Use of corticosteroids has been associated with increased skeletal muscle amino acid metabolism and thus has been believed to be due to increased ammonia production.60 However, decrease in glutamine synthetase levels, carbamoyl-phosphate synthase 1,
ornithine transcarbamylase (OTC), arginosuccinate synthase 1 and arginosuccinate lyase have also been implicated. Imoto et al also looked at the case series of use of corticosteroids in those with OTC deficiency and found a high rate of mortality with mean ammonia 761 μmol/L (ranging from 233 μmol/L to 3039 μmol/L), well above the intervention threshold (see table 5). Due to the effect on a number of key UCD enzymes, the authors recommend early intervention with renal replacement therapy and certainly close monitoring of ammonia in this group post corticosteroid intervention.

Of historical interest is Reye’s syndrome, a viral (varicella and influenza most common) induced acute liver failure due to mitochondrial injury; this was associated with high morbidity and mortality. The risk was reported to be increased in those under 12 years when aspirin was used to control fever, which led in turn to recommendation not to use aspirin in those age groups during viral infection. Reye’s syndrome peaked in the late 1970s, early 1980s and is now quite rare. It is best viewed as multifactorial, including viruses, however some cases also had an underlying IMD, mainly UCD or fatty acid disorder of oxidation.

Combination therapy of VPA plus other AEDs (such as phenytoin, phenobarbital, carbamazepine and/or topiramate, zonisamide) has also been shown to be associated with increased risk of HA. While the mechanisms for HA are clear in some, others remain uncertain and so clinicians should be aware of the increased risk of HA in combination therapy particularly in those with symptoms associated with HA.

**CLINICAL PRESENTATION**

HA can present with a number of non-specific features with guidelines suggesting that checking of ammonia should be considered in any patient presenting with acute/intermittent neurological/psychiatric presentation, acute liver failure or in the differential of sepsis with a view to making a UCD diagnosis.

Most patients present after a catabolic trigger, such as intercurrent infection, postpartum, vomiting and diarrhoea, relevant medication or a high protein meal.

In all ages, loss of appetite and vomiting are common, but in adolescents/adults the encephalopathy, hallucinations or psychiatric symptoms or signs of tremor, seizures predominate. Often these clinical features are mistaken for meningitis, brain tumour or intoxication, hence the heightened testing for checking ammonia in such clinical scenarios to avoid delay in diagnosis and adverse clinical outcomes. Around 50% of those over 16 years/adults with UCD can present acutely and mortality of up to 10% has been noted with approximately 90% requiring intensive care admission.

**LABORATORY INVESTIGATIONS**

Abnormal ammonia levels in adults should trigger further investigation with a particular focus on liver disease or a treatable IMD. Early engagement with a metabolic physician is recommended, where chronic liver disease is unlikely, in order to help expedite testing and facilitate acute management. First evaluations include: liver enzymes, arterial or venous acid-base status, renal function and electrolytes.

Low blood urea, normal glucose with a high ammonia and respiratory alkalosis increases the likelihood of UCD. The low urea could be due to UCD or protein restriction, however it should be noted that this is not a very sensitive marker and a normal urea should not be used to rule out UCD. In this context, urgent metabolic testing of urine organic acids, plasma amino acids and acylcarnitines, should be undertaken within 24 hours.

The urine organics acids are used to identify orotic acid, uracil in OTC deficiency, as well any evidence for an organic aciduria or dicarboxylic aciduria, glycines; in the case of a fatty acid disorder of oxidation. The plasma amino acids in the case of UCD are used to determine underlying UCD. In general, glutamine is elevated in UCD, and normal in organic aciduria and fatty acid disorder of oxidation. Citrulline would be low in OTC and proximal UCD, but elevated in those distal to OTC (see figure 1). A high anion gap metabolic acidosis is indicative of organic aciduria. (For more detailed discussion on interpreting relevant metabolic tests see reference 66).

As there is no specific test to confirm that HA is secondary to a specific medication, ruling out an IMD with next-generation sequencing panel testing is usually undertaken as outlined in the relevant guideline and other sources.

**ANALYSIS OF PLASMA AMMONIA**

Although historically there have been a wide variety of laboratory methods, indirect enzymatic methods that measure either nicotinamide adenine dinucleotide phosphate (NADPH) or nicotinamide adenine dinucleotide (NADH) reduction at 340 nm are most frequently used. table 4 lists some of the relevant preanalytical factors including method and effect on ammonia. By far the most significant is the delay to transfer and separation from red cells as the concentration in red blood cells is approximately three times that of plasma and represents potential for ammonia contamination (table 4). This is one of the main reasons for using plasma as the sample of choice rather than serum. Ice is used by some as a stopgap measure to reduce in vitro ammonia formation, in the absence of a suitable blood tube additive inhibitor. However, if sample handling or processing is prompt, the benefits of using ice are insignificant (table 4). The ammonia content range in 24-hour urine collection has been estimated at 4–24 mmol/L, however it is not widely available and so despite inaccuracies, underestimated use of urine/osmolal gap persists.

There is a huge degree of variability between studies and different assays in terms of time cut-offs, compounded by a lack of a robust analytical performance specification. In the introduction we used the methionet definition of HA (>40 μmol/L, 68.12 μg/L). However, to illustrate the importance of considering the laboratory method, a recent Wales External Quality Assurance (WEQAS) ammonia return with an overall method mean of 41 μmol/L, had a method range of 23–50 μmol/L which would impact on diagnosis.

Similarly, another recent WEQAS ammonia EQA return with an overall mean of 79 μmol/L, had a method range of 61–102.1 μmol/L, which is also likely to impact on treatment choice.

One of the few studies to assess biological variation in healthy individuals showed a within-individual coefficient variation of approximately 14%, within-group coefficient of variation of approximately 17% and a reference change value of 43%.

**TREATMENT**

There are no evidence-based guidelines to deal with drug-induced HA and no specific tests. There is a variety of different approaches including the Australian guidelines for managing asparaginase-induced HA; guidelines proposed by Boilèве et al for 5-FU-induced toxicity and expert panel recommendations on prevention and management of asparaginase/pegasparginase-associated toxicities in adults and older adolescents.

Best practice

Table 4  Ammonia preanalytics, patient groups and laboratory method

<table>
<thead>
<tr>
<th>Condition</th>
<th>Patients</th>
<th>Method</th>
<th>Effect on mean plasma ammonia (µmol/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power grip variable intensity for 15 mins</td>
<td>Hospital</td>
<td>Seligson diffusion</td>
<td>No effect in controls until high intensity (96 µmol/L), effect on cirrhotics increased across all levels, 30µmol/L to 87 µmol/L</td>
<td>91</td>
</tr>
<tr>
<td>Sweat</td>
<td>Healthy</td>
<td>Seligson diffusion</td>
<td>10–50 times blood ammonia-sweet ammonia increasing with decreasing sweat pH</td>
<td>92</td>
</tr>
<tr>
<td>Smoking tobacco</td>
<td>Hospital</td>
<td>Ion-exchange/phenol</td>
<td>After 1 hour an increase of 10 µmol/L</td>
<td>93</td>
</tr>
<tr>
<td>Capillary versus venous sample</td>
<td>Hospital</td>
<td>Roche Cobas Bio with Glutamate</td>
<td>Increase in capillary of 74 µmol/L compared with venous plasma of 18 µmol/L</td>
<td>94</td>
</tr>
<tr>
<td>Platelet-rich versus platelet-poor sample</td>
<td>Hospital</td>
<td>Roche Cobas Bio with GLDH Monotest (Boehringer Manheim)</td>
<td>Platelet-rich plasma ammonia of 34 µmol/L versus platelet-poor plasma ammonia of 21 µmol/L</td>
<td>94</td>
</tr>
<tr>
<td>Temperature over 90 min</td>
<td>Healthy</td>
<td>Enzymatic method</td>
<td>Mean rates of increase at 0°C, 20°C and 37°C, were 3.9 µmol/L, 5.2 µmol/L, and 25.2 µmol/L per hour, respectively</td>
<td>95</td>
</tr>
<tr>
<td>Platelet, erythrocyte, alanine transferase and gamma-glutamyl transferase</td>
<td>Healthy</td>
<td>Enzymatic method</td>
<td>Positive correlation</td>
<td>95</td>
</tr>
<tr>
<td>Tourniquet and clench</td>
<td>Hospital</td>
<td>Technicon RA-XT (Bayer Diagnostics, Basingstoke, UK) GLDH (Sigma diagnostics, Poole UK).</td>
<td>Increase of 60–75 µmol/L</td>
<td>96</td>
</tr>
<tr>
<td>Effect of hepatic dysfunction</td>
<td>Hospital</td>
<td>An enzymatic–Ultra violet kit from Thermo Electron Corporation (Infinity) was used on our AU640 analyser (Olympus UK Ltd, Hertfordshire) versus (Vitros 250, Ortho-Clinical Diagnostics, UK).</td>
<td>Due to one step enzymatic may over estimated ammonia due to NADH consumption, leading to a positive interference which can be up to threefold difference and likely to influence clinical practice</td>
<td>97</td>
</tr>
<tr>
<td>Haemolysis and Haemolysis Index cut-off</td>
<td>Unclear</td>
<td>Roche Integra 800</td>
<td>Haemolysis did not add anything over delay in time to separation</td>
<td>98</td>
</tr>
<tr>
<td>Temperature including ice, chill centrifuge versus room temperature</td>
<td>Healthy</td>
<td>AU2700 analyser (Beckman Coulter) using Randox Ammonia Reagent GLDH enzymatic (Randox, Crumlin, Co, Antrim, UK)</td>
<td>Due to one step enzymatic may over estimated ammonia due to NADH consumption, leading to a positive interference which can be up to threefold difference and likely to influence clinical practice</td>
<td>99</td>
</tr>
<tr>
<td>Smoking cannabis</td>
<td>Hospital</td>
<td>Colorimetric assay kit (BioVision Inc., Milpitas, CA)</td>
<td>After 90 min an increase of 35 µmol/L was seen</td>
<td>100</td>
</tr>
<tr>
<td>EDTA versus Li-Hep versus oxalate</td>
<td>Healthy</td>
<td>Roche COBAS c501 GLDH</td>
<td>EDTA was more precise and stable once separated- 0.322 µmol/h at 4°C, 0.122 µmol/hour at −14°C, and negligible change at −70°C</td>
<td>89</td>
</tr>
<tr>
<td>Protein of 30 g and 60 g intake after 2–3 hours</td>
<td>Healthy</td>
<td>PocketChem BA blood ammonia, microdiffusion (Lancashire, United Kingdom)</td>
<td>Maximum mean increases of 54 µmol/L for 30 g at 2 hours and 71 µmol/L for 60 g at 3 hours</td>
<td>90</td>
</tr>
</tbody>
</table>

approaches include stopping, dose reduction or continuing with measures to deal with HA. If it is decided to continue the medication then close monitoring is required and testing for other causes should be undertaken, the urgency for which is dependent on the severity of the clinical symptoms and progression of ammonia.

Stopping the offending drug suspicious for HA if possible and supportive treatment is an essential first step in management. Some cohort studies have explored this using valproate and supportive treatment is an essential first step in management. These patients are also at risk of hyponatraemia becomes encephalopathic or ammonia is increasing despite therapeutic interventions should be guided by both clinical examination and blood tests is recommended if the patient presents with ammonia >200 µmol/L, this would mean in cirrhosis, lactulose is not recommended for management of HA.

A metabolic physician should be consulted immediately once the diagnosis of HA has been confirmed. HA associated with symptoms must be treated as soon as possible. From the authors’ experience the initial steps will usually involve intravenous glucose plus ammonia scavengers (see tables 5 and 6). Any underlying dehydration will require 0.9% sodium chloride (normal saline) and if the patient is shocked or obviously unwell then admission to a high density care or intensive care unit should be arranged. Regular assessment with Glasgow coma scale (GCS), blood gas (pH, electrolytes), ammonia is routinely recommended (see tables 5 and 6). However a full updated clinical examination and blood tests is recommended if the patient becomes encephalopathic or ammonia is increasing despite initial therapy. These patients are also at risk of hyponatraemia and hypokalaemia.

With recent adult cohort data suggesting adverse neurologi- cal outcomes with ammonia values >200 µmol/L, this would suggest that more aggressive therapy (haemodiafiltration) should be considered earlier at this value. Ultimately however therapeutic interventions should be guided by both clinical presentation and ammonia level as occasionally even patients with ammonia >200 µmol/L maybe asymptomatic (tables 5 and 6). A coma for more than 3 days, raised intracranial pressure and ammonia >1000 µmol/L are particularly poor prognostic factors.

Sodium benzoate and sodium phenylbutyrate (the precursor of the active agent phenylacetate) promote ammonia removal from the body by combining with glycine to form hippurate; and with
glutamine to form phenylacetylglutamine which are excreted in urine. This alternative pathway reduces the amount of NH₃ presented to the urea cycle for detoxification. In theory each mole of benzoate removes one mole of ammonia and for phenylbutyrate this is two moles of ammonia, however due to variability in pharmacokinetics and pharmacodynamics, this is not achieved. However, such scavengers will not work adequately if hepatic function is compromised.

Dietary management of acute decompensation is crucial to prevent or stop catabolism and to correct biochemical abnormalities and ensure adequate nutritional intake. Protein intake should be reduced or paused completely. Adequate amounts of calories should be provided either enterally or parenterally (10%–20% dextrose and 20% fatty acids solutions with appropriate electrolytes: Na⁺, K⁺). This will require input from an experienced metabolic dietitian as prolonged restriction for greater than 48 hours can lead to catabolism and worsening in HA.

Haemodialysis is the most effective therapy for HA and may serve as a rescue therapy. Intermittent haemodialysis achieves the highest ammonia clearance, however due to patient stability or raised intracranial pressure continuous veno-venous haemodialysis may be more suitable. Most of the evidence around use of dialysis in HA is based on paediatric rather than adult based studies.

Liver transplantation is the definitive cure for cirrhosis and for urea cycle defects. It is considered the last resource for patients with recurrent decompensation and poor response to conventional therapies, but these are high-risk situations and require careful discussions if the patient’s condition allows.

<table>
<thead>
<tr>
<th>Ammonia level (μmol/L)</th>
<th>Action</th>
<th>Investigations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased above normal limit but ≤100</td>
<td>Reduce/stop protein intake, give intravenous glucose 6 mg/kg/min+insulin*</td>
<td>Monitor ammonia blood levels every 3 hours</td>
</tr>
<tr>
<td>&gt;100 and &lt;250</td>
<td>Start intravenous L-arginine and sodium benzoate, carbamylglutamate, carnitine, vitamin B₁₂, biotin, Correct electrolytes and phosphate</td>
<td>Monitor ammonia, electrolytes and phosphate</td>
</tr>
<tr>
<td>≥250</td>
<td>As above Avoid repetitive drug boluses Begin haemodialfiltration if no rapid drop of ammonia within 3–6 hours</td>
<td>As above Monitor supplement early especially during haemodialysis</td>
</tr>
</tbody>
</table>

There are a number of case reports/cohort studies suggesting the use of carnitine or L-arginine supplementation in the management of valproate-induced HA.

### CONCLUSION

It is important to measure ammonia in any patient of any age with encephalopathy, as early treatment can reverse the neurological deterioration or prevent death. Clinicians treating patients with the medications listed in this paper should be aware of the possibility of drug-induced HA. A greater awareness by clinicians of the importance of early diagnosis of HA and late-onset UCDS should help reduce the risk of life-threatening complications.

Detailed medical history and laboratory tests are essential to establish diagnosis. The main role of laboratory testing is to identify liver disease and IMD. Eliciting relevant clinical factors on onset HA. However, in those with confirmed HA, urgent contact with a metabolic physician should be undertaken as more specific therapies as listed above maybe required.

### References

Best practice


