MetBioNet urine amino acid requesting: a survey of current practice

Ann Bowron¹ Laura Tooth² Rachel Carling³
¹Dept of Clinical Biochemistry, Bristol Royal Infirmary, Bristol; ²Dept of Clinical Biochemistry, St Georges Hospital, London; ³Biochemical Sciences, GSTs Pathology, St Thomas’ Hospital, London

Background
A recent audit of urine amino acid (UAA) requesting at the Evelina London Children’s Hospital showed that most requests were by non-specialists and were inappropriate [1]. The lack of published standards made it difficult to establish evidence-based protocols for UAA requesting, however the audit made reference to the 2006 report from the MetBioNet amino acid working group. This group performed a review of all amino acid disorders and determined which required UAA for diagnosis, recommending that the test be performed in only a specific group of patients [2].

Establishing clear guidelines for the investigation of inherited metabolic disease (IMD) and providing advice on best practice could assist Clinical Biochemistry Departments, particularly non-specialist laboratories, in demand management and avoid unnecessary requesting of UAA analysis. Evidence of demand management is a CPA/UKAS requirement [3]. It is also important that MetBioNet laboratories demonstrate a consistent approach in light of specialist commissioning arrangements.

In view of these findings, information on the current practice in metabolic laboratories in the UK and Ireland was sought with the aim of establishing a consensus of opinion.

The survey
MetBioNet stakeholders agreed to perform a review of current practice and to provide data over a 6-month period between July and December 2012. A survey was prepared, peer-reviewed and sent to 19 MetBioNet laboratories and to all participants in the UKNEQAS UAA scheme. Responses were received from 15 MetBioNet and 3 additional laboratories.

Response to the survey
The data obtained from the survey is presented in detail below.

The responses demonstrated a varied range of practices and widely differing opinions of the value and use of UAA analysis. These differences did not correlate with laboratory size, workload or MetBioNet stakeholder status. The responses also highlighted the limitations of the questionnaire, with some confusion over the questions particularly those where opinions were sought.

There was overwhelming agreement that UAA should be analysed to investigate patients with renal stones and renal tubular disease (both primary and secondary to other IMD including mitochondrial disease). Some respondents suggested specific conditions that in their opinion require UAA analysis including hypophosphatasia and sulphite oxidase deficiency.

There was, however, considerable difference in practice and opinion on the use of UAA analysis in the investigation of other IMDs; some respondents stated it was “an essential investigation” whereas others screen all requests and do not perform the analysis unless the request is for one of the disorders outlined above.
Some additional points of interest from the survey:

- The responses demonstrated that a high proportion of requests were accompanied by clinical information. However, some respondents stated that the information provided was not of sufficient quality to advise on appropriate selection of investigations.
- Two cases of homocystinuria were missed by UAA analysis during the 6-month period studied, confirming previous findings that urine homocystine shows poor diagnostic sensitivity for this disorder [4].
- One lab reported use of UAA to identify an unusual dietary pattern which initially was masked as an IMD.
- Approximately 50% of respondents have produced in-house protocols to demand manage UAA requests.
- Those laboratories who vet requests perform significantly fewer UAA analyses and have more requests with clinical details than those which do not vet them.
- One lab reported a reduction in workload of > 90% following the introduction of a demand management protocol incorporating a fine for requestors who do not provide clinical information.
- Irrespective of whether a laboratory vetted requests, on average urine organic acids were co-requested in 75% of cases (median 75, range 49-100%)

Conclusions

There was an excellent response to the survey which may reflect both the interest in this subject and the need for laboratories to manage their workload. Where demand management protocols have been introduced, they have been effective.

There was consensus of opinion that UAA is a useful diagnostic test for the following disorders: renal stones, renal tubular disease, lysinuric protein intolerance, Hartnup Disease, sulphite oxidase deficiency and hypophosphatasia.

There was no consensus of opinion on the use of UAA as a first line screening test for the investigation of IMD which may in part be due to the ambiguity of the questions used in the survey. Concern that rejecting a request for urine amino acid analysis could potentially result in a missed diagnosis, albeit in a very small number of cases, may have led to some respondents agreeing that UAA should be a first line test for the investigation of an IMD.

Establishing and communicating a clear protocol for the investigation of IMD and educating users prior to the introduction of demand management protocols will be key to success. With the introduction of specialist commissioning a more unified approach amongst MetBioNet laboratories is desirable.

Further work

For those laboratories that chose to implement a UAA demand management programme, MetBioNet will endeavour to coordinate a common approach by preparing a set of guidelines, based on the data and experience of laboratories who have already adopted this approach.
These guidelines will:

- Include a list of conditions where UAA is considered to be an essential/recommended investigation.
- Clarify the minimum recommended investigations for a first line screening test for IMD.
- Include a statement for use when rejecting a UAA request, outlining the caveats and the need for good clinical information.
- Be summarised in a format suitable for educating users.
- Be used as a framework against which UAA requests can be audited in future.

References


Acknowledgements

Thank you very much to all who helped with preparation of the survey and to everyone who completed it with such enthusiasm.

Appendix 1.  MetBioNet recommendations for urine amino acid analysis.

Urine amino acid (UAA) analysis is recommended in the investigation of the following disorders:

- Primary renal tubular disease including cystinuria, lysinuric protein intolerance, Hartnup disease.
- Renal tubular dysfunction secondary to another inherited metabolic disorder (IMD) including mitochondrial disease
- Sulphite oxidase deficiency and molybdenum cofactor deficiency
- Hypophosphatasia

Demand management of UAA requesting.

Urine amino acid analysis is not recommended as a first line investigation for suspected IMD. From X/XX, all requests for UAA which are not accompanied by appropriate clinical details or do not fulfil the above criteria will be rejected with the following comment:

*Urine amino acid analysis is not recommended as a first line investigation for inherited metabolic disease. This sample will not be analysed and will be stored for X weeks. Please contact the laboratory on XXXXXXXXXX to discuss if analysis is clinically indicated.*
Appendix 2. MetBioNet urine amino acid requesting: A summary of responses to the survey

General
18 laboratories responded to the survey; 15 of 19 MetBioNet laboratories (79%) and 3 non-MetBioNet laboratories. All laboratories stated that they performed urine amino acids (UAA) analysis.

Processing requests for urine amino acid analysis

Q3. Does your laboratory vet requests for urine amino acid analysis?

8 Yes
10 No

Q4. What criteria do you use to vet requests?

Of the 8 labs that vet requests:
- 6 labs use clinical details (i.e. transport disorders, renal stones)
- 1 lab uses the creatinine cut off
- 1 lab uses the creatinine cut off plus a negative nitrite test

Of the 10 labs that do not vet requests:
- 1 lab review all clinical details to assess for appropriateness
- 1 lab does not offer UAA as a first line test except for a transport disorder

Q5. Do you have a creatinine cut off below which you would reject a sample?

7 labs with cut offs/caveats said the assay would be run anyway due to a chance an abnormality would be detected.
Q6. Are there any additional screening tests performed routinely on all requests for urine amino acids?

None

- Dipstick
- Nitroprusside
- Sulphitest
- Reducing Substances
- Urine Organic Acids
- GAGs

Q7. Are there any other investigations routinely added to requests for urine amino acids?

No

- Organic Acids
- GAGs
- Plasma amino acids
- Plasma homocysteine
- Urine Organic Acids
- Urine purines/purine nucleotides
- Cystine nitroprusside
- Multistix
- Sulphocystine
- Creatine
- Urate
- Galactitol

Q8. What method of analysis does your laboratory use for urine amino acids?

Screening:
- 6 labs run TLC as a screening method
- 1 lab uses ‘Waters MassTrak Amino Acids Kit (derivatisation with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate followed by UPLC separation & UV detection’
- 10 labs do not perform a screening test prior to quantitation (2 stated they had recently stopped TLC as a screening method)

Quantitative:
- 17 labs use Ion Exchange for urine amino acid analysis
- 1 lab refers samples to another lab for quantitation
Q9. What criteria are used to refer samples for quantitation from a screening test?

<table>
<thead>
<tr>
<th>Number of Labs</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/A</td>
</tr>
<tr>
<td>Abnormal TLC</td>
</tr>
<tr>
<td>Conversion to urine creatinine</td>
</tr>
<tr>
<td>Clinical Information</td>
</tr>
<tr>
<td>Nitroprusside specifically requested</td>
</tr>
</tbody>
</table>

Q10. Which automated instrument does your lab use for urine amino acid analysis?

<table>
<thead>
<tr>
<th>Number of Labs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochrom</td>
</tr>
<tr>
<td>JTEOL</td>
</tr>
<tr>
<td>Not Specified</td>
</tr>
<tr>
<td>Referred Elsewhere</td>
</tr>
</tbody>
</table>

Urine amino acids request data
Results from labs which vet requests and those which don’t are presented separately

Q11. How many requests for urine amino acid analysis did you receive between July and December 2012?

<table>
<thead>
<tr>
<th></th>
<th>Labs which vet requests (n=8)</th>
<th>Labs which do not vet requests (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>118</td>
<td>585</td>
</tr>
<tr>
<td>Range</td>
<td>15 - 723</td>
<td>313 - 1000</td>
</tr>
</tbody>
</table>

One lab which vets requests did not include requests for urine cystine.
Q12. If you provide an initial screening test how many samples are referred for quantitative analysis?

Labs which vet requests:
- 2/8 offer TLC first line and referred 2 and 23% of samples for quantitation respectively.
- 4/8 labs offer no screening test and performed quantitative analysis on 100% of samples.
- 2/8 labs did not offer a screening test and referred 13 and 48% of samples for quantitation respectively based on clinical details.

Labs which do not vet requests:
- 4/10 offer TLC first line and referred 5 – 13 % samples for quantitation.
- 6/10 offer no screening test and performed quantitative analysis on 100% of samples.

Q13. How many requests for urine amino acid analysis were accompanied by clinical details?

<table>
<thead>
<tr>
<th></th>
<th>Labs which vet requests (n=8)</th>
<th>Labs which do not vet requests (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>93%</td>
<td>87%</td>
</tr>
<tr>
<td>Range</td>
<td>77 - 100%</td>
<td>35 - 90%</td>
</tr>
</tbody>
</table>

Q14. For those requests with clinical details, how may were for the following:

<table>
<thead>
<tr>
<th>Request Type</th>
<th>Labs which vet requests (n=6)</th>
<th>Labs which do not vet requests (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic screen/?IMD</td>
<td>Median 65% Range 32 - 100%</td>
<td>Median 78% Range 72 - 91%</td>
</tr>
<tr>
<td>Renal stones/cystinuria</td>
<td>10% Range 0 - 29%</td>
<td>17% Range 5 - 32%</td>
</tr>
<tr>
<td>Renal tubular dysfunction</td>
<td>8% Range 0 - 21%</td>
<td>3% Range 0 - 8%</td>
</tr>
<tr>
<td>Other</td>
<td>12% Range 0 - 25%</td>
<td>1.20% Range 0 - 6%</td>
</tr>
</tbody>
</table>

Q15. What percentage of urine amino acids requests are from your own Trust?

<table>
<thead>
<tr>
<th></th>
<th>Labs which vet requests (n=8)</th>
<th>Labs which do not vet requests (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>59</td>
<td>25</td>
</tr>
<tr>
<td>Range</td>
<td>13 - 100%</td>
<td>6 - 100%</td>
</tr>
</tbody>
</table>

Q16. How many of the urine amino acid requests also had a urine organic acid requested?

<table>
<thead>
<tr>
<th></th>
<th>Labs which vet requests (n=7)</th>
<th>Labs which do not vet requests (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>71</td>
<td>75</td>
</tr>
<tr>
<td>Range</td>
<td>47 - 100%</td>
<td>49 - 95%</td>
</tr>
</tbody>
</table>
Q17. How many requests also had a plasma sample for amino acids requested?

<table>
<thead>
<tr>
<th></th>
<th>Labs which vet requests (n=7)</th>
<th>Labs which do not vet requests (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>60%</td>
<td>51%</td>
</tr>
<tr>
<td>Range</td>
<td>1 - 67%</td>
<td>22 - 75%</td>
</tr>
</tbody>
</table>

**Diagnostic information**

**Q18. How many urine amino acids were diagnostic of an IMD other than cystinuria?**

9 labs reported no diagnoses. The other labs reported identification of abnormal UAA in the diagnosis of the following (1 case of each unless otherwise stated):

- OTC deficiency*, CPS1, Citrullinaemia* and 4 other cases of UCD*
- intermittent MSUD*
- NKH
- Homocystinuria* and MTHFR deficiency*
- Hypophosphatasia
- Fanconi of unknown aetiology
- Imminoglycinuria (benign/incidental finding)
- Hartnup
- A sibship with increased taurine excretion due to inveterate Red Bull drinking.
- Generalised amino acid excretion in 20 patients with suspected mitochondrial dysfunction

(*respondents stated that diagnosis was made by other investigations eg plasma AA or OA and that UAA was not the diagnostic test)

**Q19. Were any amino acid results normal in samples from patients subsequently diagnosed with an IMD?**

12 labs reported no diagnoses of IMD in patients with normal UAA, many qualifying this with ‘as far as we know’.

4 labs reported abnormal UAA findings in patients with various organic acid disorders.

2 labs reported homocystinuria with no homocystine detected on UAA analysis (1 CBS deficiency missed by amino acid analyser, 1 MTHFR missed by TLC).

**Q20. To what extent do you agree with the following statement: Urine amino acids should be a first line test for the investigation of IMD?**
**Q21. Do you believe that undertaking urinary amino acid analysis adds to other tests performed in the investigation of IMDs?**

![Bar chart showing responses to Q21]  

**Q22. Under what circumstances should a metabolic laboratory perform urine amino acid analysis?**

![Bar chart showing different scenarios]  

**Q23. Additional comments**

Lab A. Forms part of the jigsaw when investigating for IMD. All evidence taken together will provide a stronger base for a proposed diagnosis. Less invasive. May not wish to collect venous samples unless absolutely necessary. Collected in community where centrifuging samples same day may be difficult. May be the only sample available, therefore need to maintain expertise in interpretation. MMA observed in organic acids, can easily check for homocystine in urine. Capacity issues if were to run plasma aas on all patients. Diagnosis of IMD relies on many complimentary investigations. A single test can provide a very good idea of the possible defect but rarely is this where the investigation ends. Provided limitations are understood, urine amino acids are a useful tool.

Lab B. Although 80% of samples came with clinical details they are in many instances not very helpful; ‘developmental delay’, ‘behavioural disturbances’ etc.

Lab C. In most circumstances, for the investigation of a suspected IMD, it is not necessary to perform urinary amino acid analysis as a first line test – this analysis can always be added at a later date based on results of the other investigations or the clinical picture.

Lab D. Although clinical details are provided for a large percentage of requests, the information given is often poor (eg ‘global developmental delay’, ‘epilepsy’, ‘?metabolic disorder’).
Lab E. We put a comment on all urine amino acid requests stating that plasma is the preferred sample for the investigation of amino acid disorders.

Lab F. Since the introduction of the revised protocol for metabolic investigations in Oct 2009, the numbers of urine samples with requests for amino acid analysis has dramatically decreased. In 2009 we received 1136 urine samples for ‘metabolic screen’ which had TLC as first line test and quantitation if abnormal or if a cystine level was required. In 2012 we received 360 samples (a decrease of 78%), of which we analysed approximately 50%, based on clinical relevance. From Jan 2013 we are imposing a charge for requests which do not supply clinical information and although early days yet, we have noticed an improvement in compliance. We rarely have been requested to retrieve a sample which was deemed unnecessary for analysis.