TMAU – diagnostic testing at Sheffield Children's Hospital.

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Introduction

The phenomenon of 'fish odour' has been reported for many centuries. More recently attributed to the tertiary amine trimethylamine (TMA), the ammoniacal body odour like the smell of rotting fish can have severely detrimental effects on the lives of those suffering from 'Fish Odour Syndrome'. Now more commonly referred to as Trimethylaminuria or TMAU, patients with this unfortunate condition exhibit increased excretion of TMA in urine as well as in sweat and breath vapour. The main causes of TMAU, low hepatic TMA oxidation and intestinal overproduction of TMA give rise to the two main types of the disorder.

The inherited form of TMAU is known as Primary TMAU (TMAU1). The result of a faulty autosomal recessive gene, TMAU1 patients have impaired activity of a liver enzyme flavin-containing mono-oxygenase type 3 (FMO3) which oxidises a wide range of substrates including many drugs. TMA is oxidised to non-odorous TMA-oxide (TMO) by FMO3 which can then be excreted. TMA itself is generated in the large intestine by bacterial degradation of compounds such as choline (high in liver, eggs and beans/peas), carnitine (meat) and TMO from seafood (TMA from fish 'spoilage' has been attributed to several species of Vitrio and Shewanella bacteria).
TMAU1 therefore results from FMO3 deficiency with an increase in the ratio of TMA to TMO in urine which can be used for diagnosis.
Due to the broad spectrum of substrates oxidised by FMO3, TMAU1 patients may suffer from adverse reactions with many drugs including codeine, tamoxifen, ketoconazole, nicotine, cimetidine, ranitidine and phenothiazine. Hypertension may result from ingestion of red wine and cheese (and chocolate), which produce the neurotransmitter tyramine, another FMO3 dependent compound. Many people suffer from migraines associated with tyramine containing foods and perhaps FMO3 deficiency may explain some of these cases, but overall this demonstrates the adverse medical consequences of TMAU1 as well as the odour related psychosocial aspects.

The acquired form of TMAU is covered by the term Secondary TMAU (TMAU2) where TMA excretion is high even though FMO3 activity is normal.
Most TMAU2 patients produce too much intestinal TMA due to excessive bacterial growth of TMA-generating species. The TMA burden is so great that FMO3 oxidation produces large amounts of TMO but (in most cases – but not all) is still unable to oxidise enough TMA to prevent an excess. This problem may be exacerbated by intestinal structural problems such as 'blind loops' or post- operative complications. TMAU2 usually presents in adulthood although children have been known to acquire excessive TMA-producing bacteria with the resultant odour.

The diagnosis of TMAU2 depends on the detection of increased urinary TMA and TMO with a normal TMA/TMO ratio indicating normal oxidation by FMO3. Patients with liver or kidney disease have been known to produce a TMAU1-like pattern of excretion, although due to a secondary cause. Importantly this may also occur with a urinary tract infection (UTI) which results in TMA being produced directly into the urine giving a false positive result. Whenever results suggest TMAU1, therefore, UTI must always be excluded by microbial analysis before a TMAU1 diagnosis can be confirmed in a follow-up sample.

Testing for TMAU

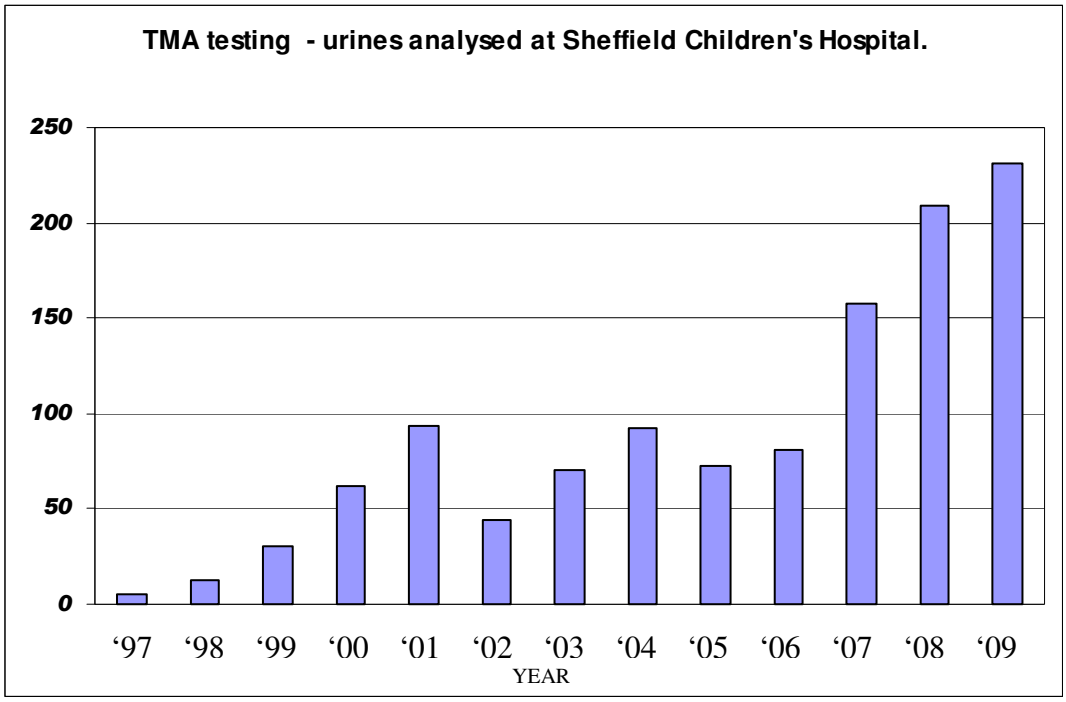
The urine test consists of two measurements:

a. trimethylamine or 'Free' TMA
b. TMA-oxide [+ free TMA] = 'Total' TMA.

The technique currently used in our laboratory is gas chromatography – mass spectrometry (GCMS) analysis of the 'headspace' vapour of heated, alkalinised urine. This method superseded a direct injection MS method used from 1997 until 2002. Results from the two methods compared well, although the current methodology allows for automated headspace sampling and GCMS injection.

A positive result is usually followed up with a routine second test after a report of the initial findings. The turnaround time for the test is currently 4 weeks or less. A GP or physician referral is essential, but we can offer advice by phone or email about how to start the process.

DNA analysis for the FMO3 gene is also now available with a turnaround time of 8 weeks. The genetic test provides the vital confirmation required for a firm diagnosis of TMAU1 and has demonstrated that the TMA / TMA-oxide ratio may normalise in TMAU1 due to spurious increases in urinary TMA-oxide.



Results and Diagnoses

We tested 1150 urines from 716 individuals from 1997 to 2009.
379 results indicated significant TMAU.
Fig. 1 shows possible (but not definitive) differentiation of TMAU1/2 on the basis of proportion of TMA compared to Total (free + oxide).

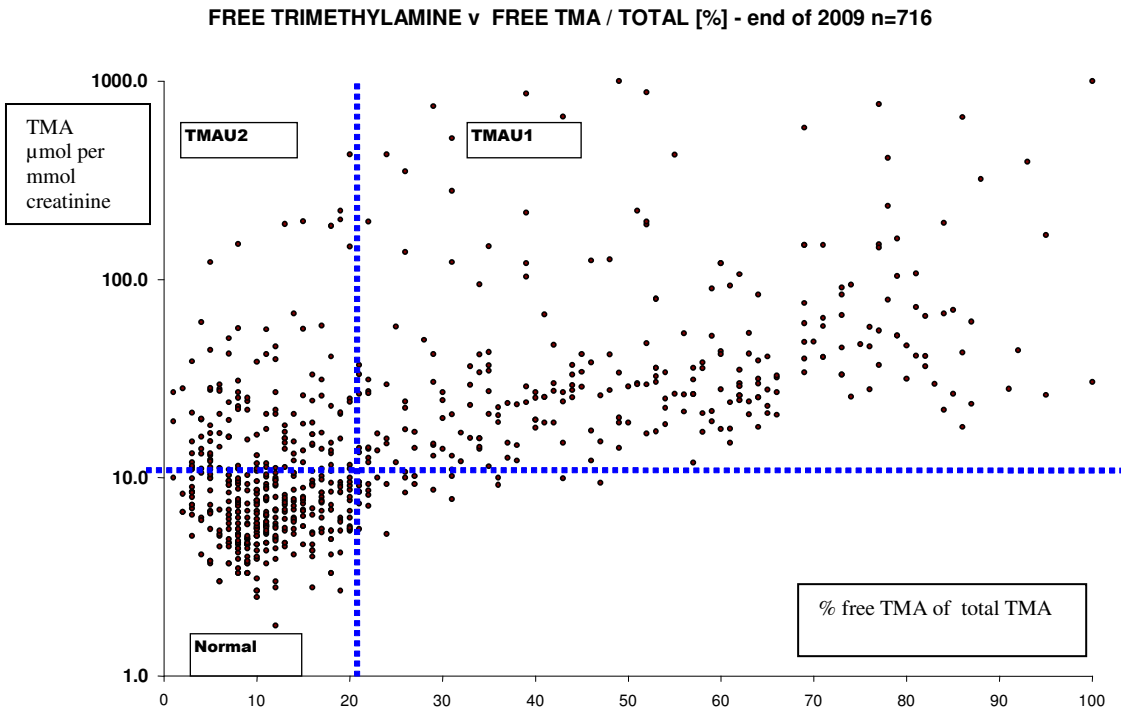


Fig. 1 Summary of samples analysed from 1998 to 2009 – TMAU1 and TMAU2 differentiation by the ratio of Free TMA to Total TMA (TMA+TMA-oxide). Free TMA normal range 1 - 11

Many TMAU sufferers may restrict their diet before testing in an effort to reduce odour. This may occasionally affect an initial diagnosis as TMA excretion may be sufficiently reduced to normal or give a normal Free to Total TMA ratio (less than 21%). For diagnostic clarity it is essential that the sample is collected when odour is at it's maximum. This may necessitate creating the conditions which induce the odour such as dietary intake of choline (eg pulses, eggs, liver), carnitine (red meat) and trimethylamine oxide (seafood). Dietary 'loading' is possibly most effective when restricted to a simple high choline meal of 2 eggs and 400g baked beans. Previously choline monohydrate was an effective loading agent but has become difficult to obtain as a chemical for patient administration. The effect of choline loading and diagnostic clarification achieved by loading can be seen in the following case report.

A case of choline load to aid diagnosis in a case of TMAU:

An adult presenting with a significant odour was tested for urinary TMA. The results showed both an increased TMA and Free/Total TMA ratio, which indicated a possible primary defect (TMAU1). [Fig.2]

A repeat 24 hour acidified sample however gave a normal Free/Total ratio, with increases in both the Free and Total TMA – suggesting the possibility of increased oxidation in response to an increased Free TMA burden (a possible indication of TMAU2).

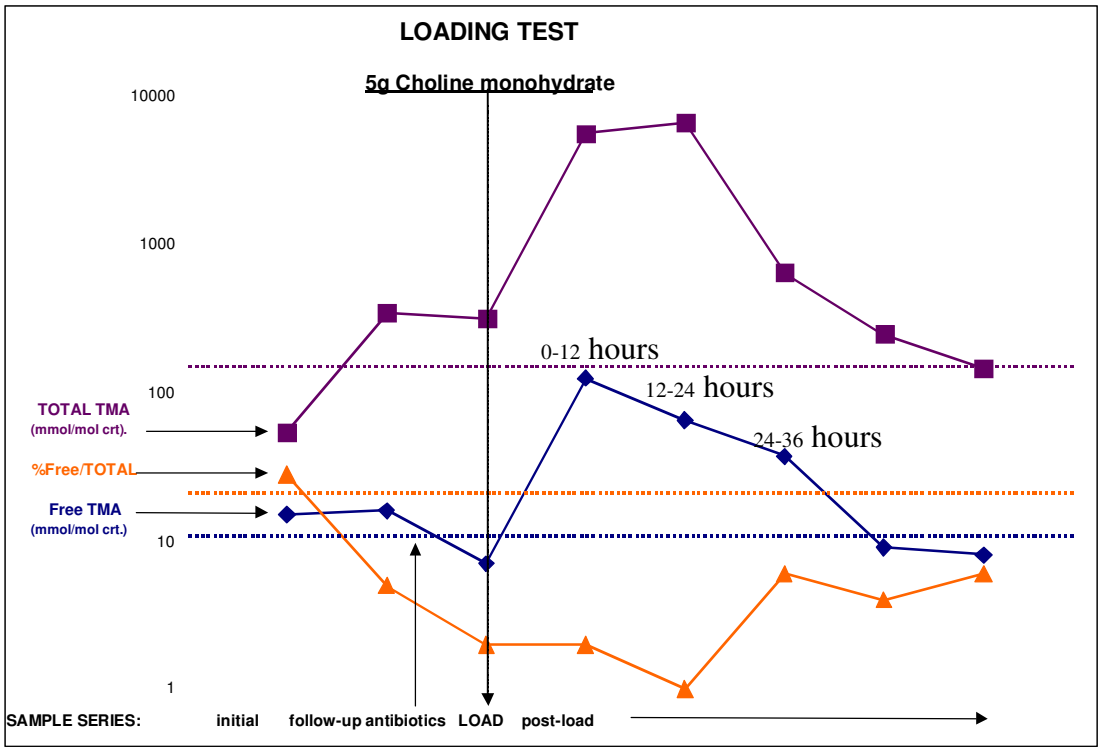
Antibiotic therapy was commenced and resulted in the normalisation of Free TMA although the Total TMA was still increased (again an indication of increased oxidation in response to increased intestinal output of Free TMA).

For clarification a 5 gram choline monohydrate load was given to the patient and samples collected for 72 hours after load.

Total TMA showed a dramatic increase (with Free TMA) and the Free/Total ratio remained within normal limits. Gradually the Free and Total TMA reduced to nearly normal excretion values.

These results indicated a treatable **TMAU2** . The first result was probably due to a urinary tract infection or bacterial contamination of the inital sample which had not been acidified). The patient was further treated with antibiotics to eradicate enterobacterial overgrowth.

Fig. 2
Dotted lines show upper limits for each parameter (nb antibiotics normalise FreeTMA at load).



Discussion

GCMS analysis of headspace vapour of alkalinised urine with stable isotope dilution provides a robust method to measure both free and total TMA for the diagnosis of TMAU1and 2. Differential diagnosis can be hampered by genito-urinary infections and intermittent presentations which may reflect TMAU1 carrier status.

Treatment of both TMAU1 and TMAU2 is based on diet to restrict the sources (precursors) of TMA and antibiotics to eliminate the TMA-producing bacteria. TMAU2 can in fact be cured by eradication of the excess bacteria, although stubborn colonies may re-grow to excess and require further courses of treatment.

TMAU1, as a genetic defect, cannot be completely cured although therapy (dietary and antibiotic) can successfully control the patient's free TMA to a less odorous level. Patient's residual enzyme activity is variable depending on the specific mutation and as such trials with the cofactor riboflavin have been tried with some success. Milder TMAU1 patients can, however, reduce their TMA to almost normal values with just diet and periodic antibiotic therapy. Other forms of therapy are based on the neutralisation of TMA chemically. Skin creams with a comparatively low pH (5.0) may neutralise alkaline TMA. This creates a non-volatile salt of TMA which lessens any odour and can be washed off by the patient later. Another solution lies in deodorising tablets such as 'activated charcoal' or copper-chlorophyllin complex (marketed as 'Nullo'). These 'internal deodorants' have been successfully used for many years and would be ideal for more severely affected TMAU1 patients.

Detection and perception of odours varies between individuals. Some people are odorous to friends, family and work colleagues but are unaware of an odour, whilst others maintain they are odorous but those around them would not agree. For those individuals who are sufficiently motivated to seek medical help, the type of odour is often difficult to describe, but ranges from 'chemical' to 'faecal'. 'Rotten fish' or 'ammonia-like' is not always mentioned, but TMAU seems to have become a focus for all malodours, possibly due to awareness of the disorder, the availability of a test and the possibility of a diagnosis.

A significant cohort of sulphurous or faecal odours have been reported by individuals who contact the laboratory. This may be another enterobacterial problem, but although Shewanella species are known to produce both hydrogen sulphide and TMA, we have yet to measure an increased TMA or TMO as a secondary marker for enterobacterial overgrowth in these cases.

For those with a significant TMAU, difficulties in diagnosis mainly stem from the interpretation of TMA and free/total ratios given the background of diet, variation of enzyme activity and variation of bacterial sources of TMA. This can be summarised by the sub-types of presentation and biochemical abnormality we have encountered over the past 12 years. The new FMO3

mutation service should help to clarify TMA results greatly in the years to come.

TMAU1 and TMAU2 possible sub-types:

a. TMAU1 transient neonatal – possible delay in switch from fetal enzyme FMO2 to FMO3 TMA oxidation, but resolves during development.
b. TMAU1 severe childhood - parenting / schooling problems are possible.
c. TMAU1 adulthood – probably presented in childhood; long-term sociopathy.
d. TMAU1 heterozygote – may present only during dietary load / menses.
e. TMAU1 very mild – 'double dose' DNA polymorphisms – TMA borderline.
f. TMAU1 FMO3 mutation proven TMAU1 with increased TMO (like TMAU2)

a. TMAU2 severe neonatal -'sepsis' massive TMA responds to antibiotics.
b. TMAU2 childhood - antibiotic eradication prevents school / social problems.
c. TMAU2 adulthood – may have long history of odour, eradication possible.
d. TMAU2 intermittent – difficult diagnosis without precursor load.
e. TMAU2 due to UTI – presents biochemically as TMAU1 (urine-only odour).
f. TMAU2 due to renal or hepatic dysfunction – presents as TMAU1.

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