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PROBLEMS OF ACYLCARNITINE ANALYSIS WHEN CARNITINE IS DEPLETED: INSIGHTS FROM THE ERNDIM QUALITATIVE ACYLCARNITINE SCHEME

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BACKGROUND

The ERNDIM qualitative acylcarnitine external QA scheme circulates dried blood spots from patients with metabolic disease detectable by acylcarnitine profiling. Samples are from real patients whose diagnoses have been confirmed and are either excess blood "left over" from diagnostic or patient monitoring, or extra blood taken with permission when the patients are routinely sampled. Informed consent is obtained from all such donors for anonymised use of blood for quality assurance purposes. 40µl aliquots of lithium heparin anti-coagulated whole blood are spotted onto Whatman 903/Alhstrom 226 paper and dried for 24h at RT before storage at –80ºC, packing and despatch. The London scheme is in its 11th year of operation. It grew from 45 participants in 2003 to 100 in 2010 when the scheme was divided between two centres, London and Heidelberg. Each now have more than 60 participants.

ACYLCARNITINE ANALYTICAL METHODS

All current participants measure blood spot acylcarnitines using positive electrospray ionisation tandem mass spectrometry (Esi—MSMS). Results reported are derived from precursor ion scans (precursors of m/z 85+), multiple reaction monitoring (MRM, SRM) of specific precursor/product pairs for selected acylcarnitine species, or a combination of both acquisition modes, typically precursor ion scans for qualitative analysis and MRM for quantitation. A questionnaire accompanying the 1st circulation in 2003 revealed that 34/35 respondents used butylation and 1/35 analysed samples underivatised. Responses to a similar questionnaire with the 19th circulation London 2012 showed 34/44 butylated whilst 10/44 analysed underivatised (23%). 15/44 in 2012 used commercial kit methods (9/34 derivatised, 6/10 underivatised). No obvious diagnostic advantage or disadvantage of butylation vs underivatised methods has been evident from the ERNDIM scheme. Notably, diagnoses requiring detection of dicarboxylic acylcarnitines e.g.GA-1 did not prove difficult for underivatised methods.

SAMPLES AND RESULTS

Samples circulated between 2003 and 2012, and the proportion of respondents correctly identifying them are listed in Table 1. There were a number of samples which posed problems for participating laboratories. Where a pattern emerged was in the subset of samples where patients exhibited secondary carnitine depletion.

Sample 13a (2009) was from a hyperammonaemic 8d old patient with propionyl CoA carboxylase deficiency (Figure 1). 55/73 respondents suggested the disorder but 18/73 did not, with 16/73 suggesting carnitine uptake disorder (CUD). The median (range) reported carnitine concentration was 4.2 (2.0-15.0)µmol/L, 0.4 fold the median lower reference interval (MLRI). C3 carnitine was 3.4 (2.2-9.4)µmol/L, 1.1 fold above the median upper reference interval (MURI) & C3/C2 ratio 1.2 (0.5-2.9), 6.1 fold above MURI.

Samples 2c (2003, Figure 2) and 19b (2012, Figure 3) were from patients with VLCADD. 16/32 respondents suggested VLCADD for sample 2c and 20/49 for sample 19b. 3/32 suggested CUD for 2c and 28/49 for 19b. The carnitine concentration of 19b was 6.3 (2.8-15.0)µmol/L, 0.5 fold the MLRI. C14:1 carnitine was 0.26 (0.18-0.34)µmol/L, 1.4 fold above MURI and C14:1/C16 ratio 0.65(0.38-0.86), 3.3 fold above MURI. 2c results were similar.

COMMENTS

It was particularly surprising that so many laboratories failed to identify the neonatally presenting propionyl CoA carboxylase deficiency sample, since extreme carnitine depletion is not uncommon in these patients. Likewise, patients with VLCADD are frequently carnitine depleted and C14:1 levels may not be grossly elevated. A high proportion of laboratories in 2009 and 2012 suggested CUD on the basis of the low carnitine. It seems possible that these reports derived from the use of individual acylcarnitine reference ranges. If rules based reporting systems are to be used it would seem prudent to incorporate appropriate ratios in the algorithms and/or to flag samples for particular scrutiny when free carnitine is low. These examples emphasise the importance of looking for disproportionately raised acylcarnitine species in the face of carnitine depletion: reference ranges cease to be valid and ratios may provide better discrimination if quantitative, objective diagnostic indicators are required.

Table 1: Circulations London 2003-2012. Samples discussed are highlighted

Diagnosis	No of samples	Primary acylcarnitine reported	% Correct diagnosis
Normal	13		Average=90
Normal CRF	1		63
MCADD	10	C8, C10:1, C6	average=99
MMA (mutase)	5	C3, C4DC	91, 94, 100, 93, 66
MMA (Epimerase)	1	C3, C4DC	49.2
MMA (Cobalamin B)	1	C3, C4DC	92.6
PA	5	C3	98, 95, 98, **75**, 100
VLCADD	4	C14:1	**50**, 95, 97, **41**
GA-1	4	C5DC	0.0, 98,89,100
IVA	2	C5	99
LCHAD	3	C18OH, C16OH, C16:1OH	92
Malonic	2	C3DC	18.0, 52.0
CTD	4	low C0	80
MADD	2	C5, C6, C8, C10. C12	61
CPT-1	1	C0, C16	95
3-MCC	1	C5OH	85
HMGCoA Lyase	1	C5OH, C6DC	79

Figure 1 Underivatised scan PA: Sample 13a

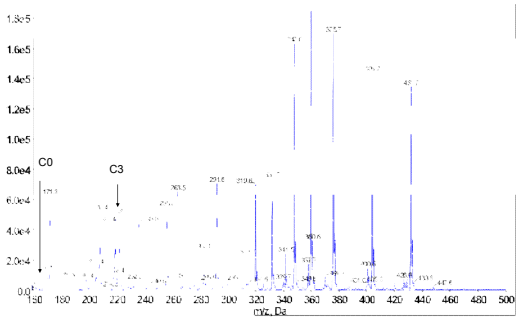


Figure 2 Underivatised scan VLCADD: Sample 2c

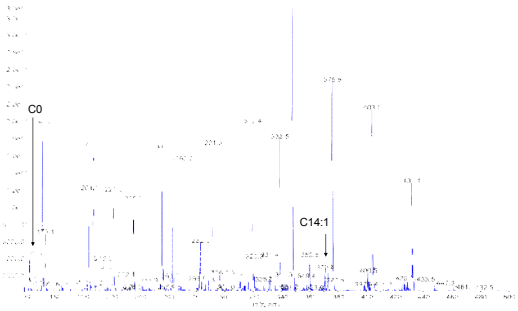
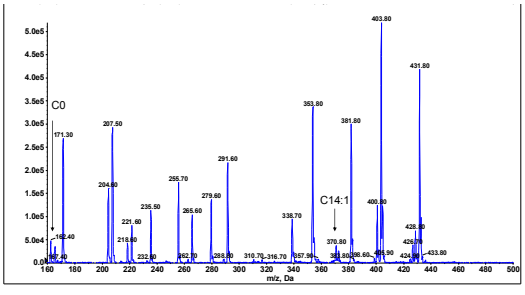


Figure 4 Underivatised scan VLCADD: Sample 19b



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