

Tandem Mass Spectrometric Analysis for Aminoacid, Organic Acids and Fatty Acid Disorders in Critically ill Newborns from Indian NICU

*Anil B. Jalan **, *Adolf Muehl ***

NIRMAN, 203, Nirman Vyapar Kendra, Sector 17, Vashi – Navi-Mumbai, India.

* Chief Scientific Research Officer (Bio – chemical Genetics)

** AKH Kinderklinik, University of Vienna, Austria.

Background : Tandem mass spectrometry (MS / MS) with two mass spectrometers, on either side of a collision cell, offers an analytical technique capable of measuring many analytes with rapid throughput per sample (approx. 2 min.). This technique also offers advantage of simple sample preparation, as the first mass spectrometer is used to separate analytes with in the sample, which are then collision cell and finally detected in the second mass spectrometer. With the recent use of electro – spray for sample introduction into the MS / MS, automation became possible, providing a technique now suited to population screening. Tandem mass spectrometry (MS / MS) is rapidly being adopted by newborn screening programs to screen dried blood spots for more than 20 markers of diseases in a single assay. Limited information is available for setting the marker cutoffs and for the resulting positive predictive values. (1). There is no Indian data available for cutoffs, especially in critically ill newborns. It is estimated that 5 % of NICU babies will show abnormal markers (1).

Methods:- A prospective study was done over a period of last 2 years. 60 Critically ill newborns 46 males and 14 females were selected with age group ranging from 0 – 90 days (mean 18.18 days). The blood spots were collected on S & S 903 filter paper and were air dried for 3 hrs. Thereafter the samples were sent to Vienna University for analysis with clinical history. The sample preparation, internal std. And measurements were done by the method described by Schulze et al (2). Instruments used were HPLC Micro – pump : Perkin Elmer 200, Auto – sampler – Gilson 215 and Mass Spectrometer : Perkin Elmer API2000. The test time was ~ 1.5 min per sample. All the patients were tested in duplicate. The parameters tested were Total Carnitine, Free Carnitine, Acyl Carnitines (C2 – C18) and 10 different Aminoacids. For the purpose of calculation of normal patients are not considered and the mean, SD and Cutoffs (Mean + 3 SD) are calculated from the data of 54 normal patients. Cut off is taken as Mean + 3 SD as it corresponds to 99th centile and is the minimum cut off acceptable.

No	Symbol	Metabolite	Mean	1 SD	M + 2SD	M + 3SD	Cut -off
1	TC	Total Carnitine	43.43	28.83	101.09	129.92	129.92
2	FC	Free Carnitine	31.56	23.61	78.78	102.38	102.38
3	AC	Acyl Carnitine	11.86	8.57	29.00	37.57	37.57
4	FC / AC	Free / Acyl Carn. Ratio	3.12	1.93	6.98	8.92	8.92
Short Chain Acyl Carnitine							
5	C 2	Acetyl Carnitine	7.88	6.15	20.18	20.34	20.34
6	C 3	Propionyl Carnitine	1.03	0.82	2.67	3.49	3.49
7	C 3 DC	Malonyl Carnitine	0.11	0.08	2.67	0.36	0.36
8	C 4	Butyryl Carnitine	0.45	1.33	3.12	4.45	4.45
9	C 4DC	Methylmalonyl Carnitine	0.19	0.11	0.40	0.51	0.51
10	C 5	Isovaleryl Carnitine	0.25	0.18	0.60	0.78	0.78
11	C 5 OH	3 Hydroxyisovaleryl Carnitine	0.18	0.15	0.48	0.63	0.63
12	C 5:1	Tiglyl Carnitine	0.17	0.14	0.45	0.58	0.58
13	C 5 DC	Glutaryl Carnitine	0.11	0.09	0.29	0.38	0.38
14	C 6	Hexnoyl Carnitine	0.19	0.17	0.52	0.69	0.69
15	C 6 DC	Adipyl Carnitine	0.13	0.10	0.32	0.42	0.42
16	C 8	Octanoyl Carnitine	0.16	0.13	0.42	0.56	0.56
17	C 10	Decanoyl Carnitine	0.29	1.05	2.39	3.43	3.43
18	C 10 : 1	Decenoyl Carnitine	0.17	0.15	0.47	0.62	0.62
19	C 10 : 2	Decadienoyl Carnitine	0.16	0.11	0.39	0.50	0.50

Tandem Mass Spectrometric Analysis for Aminoacid, Organic Acids and Fatty Acid Disorders in Critically ill Newborns from Indian NICU

*Anil B. Jalan *, Adolf Muehl ***

NIRMAN, 203, Nirman Vyapar Kendra, Sector 17, Vashi – Navi-Mumbai, India.

* Chief Scientific Research Officer (Bio – chemical Genetics)

** AKH Kinderklinik, University of Vienna, Austria.

20	C 12	Lauroyl Carnitine	0.33	0.35	1.02	1.36	1.36
21	C 14	Myristoyl Carnitine	0.28	0.23	0.74	0.96	0.96
22	C 14:1	Tetradecenoyl Carnitine	0.19	0.17	0.54	0.71	0.71
23	C 14:2	Tetradecadienoyl Carnitine	0.17	0.13	0.44	0.58	0.58
24	C 16	Palmitoyl Carnitine	1.05	0.86	2.76	3.62	3.62
25	C 16OH	Hydroxypalmitoyl Carnitine	0.06	0.04	0.13	0.17	0.17
26	C 16 : 1	Hexadecenoyl Carnitine	0.43	0.33	1.10	1.43	1.43
27	C 18	Stearoyl Carnitine	0.32	0.22	0.77	1.00	1.00
28	C 18 OH	Hydroxystearoyl Carnitine	0.08	0.08	0.24	0.31	0.31
29	C 18:1	Oleyl Carnitine	0.47	0.29	1.05	1.34	1.34
30	C 18:1- OH	Hydroxyoleyl Carnitine	0.08	0.07	0.22	0.29	0.29
31	C 18:2	Linoleyl Carnitine	0.16	0.13	0.42	0.55	0.55
32	C 18:2 - OH	Hydroxylinoleyl Carnitine	0.21	0.11	0.44	0.55	0.55
33	Ala	Alanine	188.35	94.68	377.70	472.38	472.38
34	Arg	Arginine	18.93	12.39	43.72	56.11	56.11
35	Asp	Asparagine	47.17	38.53	124.23	162.76	162.73
36	Cit	Citrulline	8.69	4.19	17.06	21.24	21.24
37	Glu	Glutamic Acid	213.35	151.26	515.88	667.15	667.15
38	Gly	Glycine	196.87	82.21	361.29	443.50	443.50
39	Leu	Leucine	132.61	55.97	244.54	300.51	300.51
40	Met	Methionine	16.30	10.07	36.45	46.53	46.53
41	Orn	Ornithine	18.97	8.75	36.46	45.21	45.21
42	Phe	Phenylalanine	43.37	19.96	83.30	103.26	103.26
43	Tyr	Tyrosine	50.97	31.34	133.66	145.01	145.01
44	Val	Valine	114.48	54.92	224.33	279.25	279.25

Conclusion:- It is now possible to screen rapidly, simultaneously and inexpensively for about 31 rare disorders with the use of Tandem Mass Spectrometry (3). We have selected Critically ill newborns, as this is the most likely group of babies who will receive MS / MS by DBS in the near future in India. The general population screening is still at horizon but screening of NICU babies is the most urgent need. We have hereby derived Mean \pm 2 SD and Cutoffs for various parameters used in MS/MS for babies admitted in NICU Critical illness. Of these 60 babies one baby had MMA (1/60), 1 GA Type II (1/60), 1 Hyper – alaninemia (1/60) 1 with UCD (1/60), 1 Hyper – methioninemia (Homocysteinuria) (1/60) and 1 was MSUD (1/60). Thus there were 6 babies (6/60) with IEM detected by MS/MS alone. Thus the pickup rate in NICU setting by MS/MS alone was 10.0% (6/60). We therefore conclude that MS/MS is a highly sensitive, economical screening test to be employed in all NICU babies and we have setup mean and cutoff levels for various parameters in Indian children.

References:-

1. Thomas H Zytковicz et al, Tandem Mass Spectrometry analysis for Amino, Organic, Fatty Acid disorders in Newborn DBS, Clinical Chemistry 47:11, 1945 – 1955 (2001).
2. Andreas Schulze et al., Accurate Measurement of Free Carnitine in Dried blood spots, Clinical Chemical Act 335 (2003), 137 – 145.
3. B. Wilcken, Kevin Carpenter et al. Screening Newborns for Inborn Errors of Metabolism by Tandem Mass Spectrometry, N Eng J Med 2003; 348: 2304 – 12.