

## Metabolic Screening by Tandem – Mass: What Should We Know

**Jorge Sales Marques\***

*Pediatric Department, Centro Hospitalar Conde S. Januário, Macau, China*

**\*Corresponding Author:** Jorge Sales Marques, Pediatric Department, Centro Hospitalar Conde S. Januário, Macau, China.

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### Abstract

Tandem mass diagnose metabolic diseases through filter paper in the neonatal period. The number of metabolic diseases included in each country's national program, depends on economic factors, increased incidence in each region or country and health policy.

The most frequent diseases screened in this period, are mainly these four groups: aminoacidopathies, urea cycle defect, fatty acid beta oxidation deficiency and organic aciduria. After the diagnosis of the disease, we can perform an early treatment to avoid complications, even sudden infant death episode particularly in fatty acid beta oxidation deficiency. We can also offer for the couple, genetic counseling and prenatal diagnosis for the next pregnancy.

**Keywords:** Tandem-Mass; Neonatal Screening

Tandem mass diagnose several metabolic diseases through filter paper during the neonatal period.

The number of metabolic diseases included in each country's national program, depends on economic factors, increased incidence in each region or country and health policy.

Is important to have reference centers for treatment in order to avoid unnecessary costs and to concentrate as many as possible, patients with these pathologies, so that medical staff can gain more experience in treatment and follow-up of these children.

The most frequently screened pathologies in many countries are divided in four groups:

1. Aminoacidopathies: classic phenylketonuria(PKU), maple syrup urine disease(MSUD), tyrosinemia type 1 and classic homocistinuria.
2. Urea cycle defect: argininosuccinic aciduria, citrullinemia.
3. Fatty acid beta oxidation deficiency: medium-chain acyl-CoA dehydrogenase (MCAD), long-chain acyl-CoA dehydrogenase (LCHAD), verylong-chain acyl-CoA dehydrogenase (VLCAD), short-chain acyl-CoA dehydrogenase (SCHAD), carnitine palmitoyltransferase 1 (CPT I), carnitine palmitoyltransferase 2 (CPT II) and primary carnitine deficiency.
4. Organic aciduria: propionic, methylmalonic, isovaleric, 3-hydroxy-3methylglutaric and glutaric type 1

The first group of diseases includes some of the aminoacidopathies:

### PKU

**Incidence:** 1/8000.

**Clinic:** Severe mental retardation, seizures, spasticity, microcephaly, eczema in 20 - 40%, hypopigmentation in the eyes, hair and skin.

**Laboratory:** High phenylalanine and low tyrosine in plasma, PAH gene for confirmation.

**Treatment:** If phenylalanine > 6 mg/dl – start treatment with protein restriction.

If phenylalanine between 6 - 15 mg/dl – reduce phenylalanine intake throughout maternal or formula milk and start a milk without phenylalanine.

If phenylalanine >15 mg/dl – stop maternal or formula milk and start a milk without phenylalanine. Increase the caloric intake by using glucose supplement and corn oil.

**MSUD**

**Incidence:** 1/185000.

**Clinic:** Hypertensive fontanelle, apnea, bradycardia, hypothermia, coma, hypertonia, opisthotonus, axial hypotonia (classic form-first week of life- leucine > 2000  $\mu\text{mol/L}$ ; Intermediate form-infant/infancy-leucine < 2000  $\mu\text{mol/L}$ ).

**Laboratory:** Plasma and urine – increase leucine, isoleucine and valine, ketoacidosis, urine odour of maple syrup. Confirmation by enzyme study – branched-chain  $\alpha$ -oxoacid dehydrogenase or gene determination: E1 $\alpha$ /BCKDHA, E1 $\beta$ /BCKDHB, E2/DBT and E3/DLD.

**Treatment:** Glucose a 10% - 8 - 15 mg/kg/min ev, add insulin 0,1 - 1 U/kg/h if glucose > 200 mg/dl, lipids - 2,5 g/kg/d, water load – 130 - 160 ml/kg/d. Restriction of leucine, isoleucine and valine. Need peritoneal dialysis, hemofiltration or exchange transfusion in case of severe neurological symptoms, clinical deterioration, leucine decrease of less than 500  $\mu\text{mol/L}$  in 24 hours.

**Tyrosinemia type 1**

**Incidence:** 1/1000000.

**Clinic:** Involve mainly the liver, kidney and peripheral nerves, stem from the cytotoxicity of tyrosine metabolites, fumarylacetoacetate, maleylacetoacetate, succinylacetoacetate and succinylacetone, accumulating proximal to the metabolic defect. Cause polyuria, polydipsia, failure to thrive, retardation of motor development, rickets.

**Laboratory:** Microcytic hypochromic anemia, hypoglycaemia, hypophosphatemia, increase levels of aminotransferases, alkaline phosphatase and alpha-fetoprotein, increase of plasma tyrosine, generalized hyperaminoaciduria, presence of succinyl acetoacetic acid and succinylacetone in urine. Skin biopsy confirm lack of the enzyme fumaryl acetoacetate hydroxylase or determination of FAH gene.

**Treatment:** Diet restricted in phenylalanine and tyrosine does not prevent progression of the liver disease and development of hepatocellular carcinoma and liver transplantation was previously the only option for these patients. NTBC, a potent inhibitor of 4-hydroxyphenylpyruvate dioxygenase, has transformed the natural history of tyrosinemia. The aim is to block tyrosine degradation at an early step so as to prevent the production of toxic down-stream metabolites. The dose of NTBC: 1mg/kg/d, bid, associated with hypoproteic, hypercaloric diet, restricted in tyrosine and phenylalanine, vitamin D, phosphate, calcium, potassium and sodium citrate.

**Classic Homocystinuria**

**Incidence:** 1/100000

**Clinic:** The newborn is normal at birth and the symptoms start at 3 year of age with inferior dislocation of the lens. Can cause also cataracts, glaucoma, optic nerve atrophy. The muscle skeletal system showed arachnodactyly of hands and feet fingers, cyphoscoliosis and marfan-like features. The most severe problem is thromboembolism phenomenon that occurs mostly in adolescents after 15 year of age.

**Laboratory:** high levels on plasma and urine of cysteine, homocysteine and methionine, determination of cystitone beta-sintetase or CBS gene.

**Treatment:** Low intake of methionine, pyridoxine (vitamin B6) – 50-100 mg/d, supplement with folic acid – 10 mg/d, hydroxocobalamin – 1mg/d oral from 5 y of age, vitamin C- 100 mg/d, acetylsalicylic acid – 5 mg/kg/d oral (maximum dose 100 mg/d) for prophylaxis of thromboembolism and use of betaine – 100 mg/kg/d, in case of non-respond to vitamin B6.

The second group is urea cycle defect:

**Argininosuccinic aciduria**

**Incidence:** 1/500000.

**Clinic:** Feeding problems, vomiting, lethargy, convulsions, hypertonia, hepatomegaly, coma.

**Laboratory:** Plasma- increase ammonia, citrulline (100 – 300 µM), argininosuccinic acid and orotic acid, with reduce arginine. Enzyme study or determination of ASL gene for confirmation.

**Citrullinemia**

**Incidence:** 1/200000

**Clinic:** Vomiting, food refusal, seizures, hypotonia, hypertonia, hypothermia, hepatomegaly, hypertensive fontanelle, trichorrhexis nodosa (fragile, breakable, dry hair), coma.

**Laboratory:** Hyperammoniemia, liver dysfunction, increase coagulation time, increase citrulline (> 1000 µM), increase glutamine and alanine, reduce arginine and increase orotic acid in the urine. Enzyme studies or ASS gene will confirm the diagnosis.

Treatment for both diseases:

1. Stop protein intake and reduce catabolism
2. First infusion (2h) with glucose 10 % - 10 mg/kg/min with appropriate electrolytes.
3. Arginine- 400 - 700 mg/kg/d
4. Sodium benzoate- 250-500 mg/kg (if possible associate sodium phenylacetate- 450- 600 mg/Kg iv or oral)
5. Control of glucose and ammonia after 2h, associate insulin 0,1 – 1 U / kg / h if glucose > 200 mg / dl
6. Peritoneal dialysis or hemofiltration – if ammonia > 500 µmol/l.

The third group are diseases of fatty acid beta oxidation:

**MCAD**

**Incidence:** 1/110000

**Clinic:** Hypoketotic hypoglycemia, myopathy, cardiomyopathy, sudden dead.

**Laboratory:** Hyperammoniemia, hyperuricemia, low carnitine. Determination of prevalent mutation p.K329E in ACADM gene.

**Treatment:** Avoid prolong fasting, glucose 5%, 8 - 10 mg/kg/min iv, carnitine- 100mg/kg /dia iv, riboflavine- 50- 200 mg/d, iv, im or oral, raw starch- 1-2 g/kg/d, start at 8 months of life. Cannot give MCT oil to these patients.

**SCHAD**

**Incidence:** 1/35000

**Clinic:** Hypoketotic hypoglycaemia caused by hyperinsulinism

**Laboratory:** Show high levels of hydroxybutyrylcarnitine (C4OH) before and after meals, that is not present in persistent hyperinsulinemic hypoglycaemia of infancy.

Organic acids profile in urine revealed an increase of 3-hydroxyglutaric acid. The molecular study of HADH gene or skin fibroblast culture with decrease of SCHAD activity, confirm the diagnosis

**Treatment:** Frequent feeding (3-3h). Supplement with maltodextrin 8 mg/Kg/d, 8h oral and diazoxide - 8 mg/Kg/d, 8h oral.

### VLCAD

**Incidence:** 1/500000

**Clinic:** Hepatomegaly, myopathy, cardiomyopathy

**Laboratory:** Hypoketotic hypoglycemia, high CK. Confirmation of the disease, is done by study of ACADVL gene.

**Treatment:** Avoid fasting, glucose 5% - 8 - 10 mg /kg / min, MCT oil - 1 - 1,5 g / kg (40 - 60 % of total lipids), carnitine - 100 mg / kg /d iv, supplement of raw corn -1 - 2 g /kg/d start at 8 months of age.

### LCHAD

**Incidence:** 1/80000

**Clinic:** Hypotonia, cardiomyopathy, hepatic dysfunction, peripheral neuropathy, pigmentary retinopathy, encephalopathy.

**Laboratory:** Ketotic hypoglycemia, hyperammonemia, high CK, low carnitine 3- hydroxycarboxylic aciduria. The disease is confirmed by study of mutation in p.E510Q in HADHA gene.

**Treatment:** Avoid prolonged fasting, MCT oil - 1-1,5 g/kg (40 - 60% of total lipids), carnitine iv - 50 - 100 mg/kg/d, riboflavine - 75-100 mg/d, supplement of raw corn - 1- 2 g/kg/d start at 8 months of age.

### CPT I

**Incidence:** 1/524287 (Baviera - Germany). Worldwide only 50 cases

**Clinic:** Convulsions, coma, hepatomegaly.

**Laboratory:** Hypoketotic hypoglycemia, high CK, normal ou high carnitine, absent of dicarboxylic aciduria, carnitine palmitoyltransferase 1 deficiency. Check for CPT1A gene for confirmation.

**Treatment:** Avoid prolonged fasting and long chain fatty acids, glucose - 8 a 10 mg/kg/min, MCT oil - 1-1,5 g/kg (40- 60 % of total lipids).

### CPT II

**Incidence:** 1/500000

**Clinic:** Cardiomyopathy, hepatic dysfunction, myoglobinuria after exercise, cold exposition, fasting and infectious diseases.

**Laboratory:** Low total carnitine. Check for CPT2 gene for confirmation of the diagnosis.

**Treatment:** Same as CPT 1 + carnitine- 100 mg/kg/d iv and supplement of raw corn - 1- 2 g/kg/d start at 8 months of age.

### Primary carnitine deficiency

**Incidence:** 1/100000; 1/40000 in Japan.

**Clinic:** Cardiomyopathy, arrhythmias, muscle weakness, liver disease.

**Laboratory:** Very low free/total carnitine ratio, all acylcarnitine's are reduce. Confirmation of the disease by SLC22A5 gene.

**Treatment:** Oral carnitine 100 - 200 mg/kg/d.

The four group are organic acids disorders:

### Propionic Aciduria

**Incidence:** 1/200000.

**Clinic:** Vomiting, dehydration, hypotonia, coma.

**Laboratory:** Ketoacidosis, neutropenia, thrombocytopenia, high levels of glycine and alanine, Hyperammonemia, low carnitine, hypoglycemia, methyl citric acids and 3-OH-propionic in urine. Confirmation of the disease determination of by PCCA and PCCB genes.

**Treatment:** Avoid long period of fasting, restriction of isoleucine, valine, methionine, threonine, glucose 10 % - 8-15 mg/Kg/min iv, carnitine- 100 mg/kg/dia iv, metronidazole - 10 - 20 mg/d and/or colistin for 10 days /month, biotin - 10- 20 mg/d.

After 48 h – start 0,5 g proteins /kg/d (Vamin iv/milk)

### **Methylmalonic aciduria**

**Incidence:** 1/60000.

**Clinic:** Vomiting, dehydration, feeding problems, growth retardation, mucocutaneous candidiasis, hepatomegaly, osteoporosis, extrapyramidal movements, progressive renal failure.

**Laboratory:** Ketoacidosis, neutropenia, thrombocytopenia, hypoglycemia, hyperammonemia, low carnitine, increase glycine and alanine. The methylmalonic, 3-OH-propionic and methyl citric acids are also increase in urine. Confirmation of the disease by determination of MUT gene.

**Treatment:** Same as propionic aciduria (but no need biotin), associate vit B12 (respond in 50 % of cases), OHCbl – 1-2 mg im /d.

### **Isovaleric aciduria**

**Incidence:** 1/250000.

**Clinic:** Vomiting, refuse to eat, dehydration, typical odor (stinky feet, mold), convulsions, intraventricular and cerebellar hemorrhage, coma.

**Laboratory:** Ketoacidosis, hyperglycemia, hypoglycemia, neutropenia, thrombocytopenia, low carnitine, isovalerylglycine and 3-hydroxyisovaleric acids in urine. Check for IVD gene for confirmation.

**Treatment:** rehydration and correction of acidosis, diet without protein, restriction of leucine, glucose 10% – 8-15 mg/kg/min iv, insulin 0,1-1 U/ kg/h if hyperglycemia, carnitine iv – 100 - 200 mg/kg/d, L- glycine – 150- 250 mg/kg/d.

### **3 -hydroxy- 3 methylglutaric aciduria**

**Incidence:** 1/100000.

**Clinic:** Vomiting, hypotonia, hepatomegaly, encephalopathy, convulsions.

**Laboratory:** Severe metabolic acidosis, hypoketotic hypoglycemia, hyperammonemia, liver dysfunction, hyperlactacidemia, increase of 3-OH-metilglutaric, 3-OH-isovaleric, 3-metilglutaconic, 3-metilglutaric acids in urine. Brain MRI shows diffuse abnormality in signal intensity of the cerebral white matter and abnormal signal intensity of the thalami and basal ganglia. Confirmation of the disease by study of HMGCL gene.

**Treatment:** Carnitine – 100 mg/kg/d, glucose iv- 8-15 mg /kg/min, restriction of lipids (25% of daily needs) and protein, avoid prolonged fasting.

### **Glutaric aciduria type 1**

**Incidence:** 1/200000.

**Clinic:** Macrocephaly, nausea and vomiting, profuse sweating, unexplained fever, irritability, insomnia, hepatomegaly, encephalitis crisis, neurodegenerative disease with spasticity, dystonia, hypotonia, choreoathetosis, ataxia, dyskinesia, retinal haemorrhage (same as shaken – baby syndrome).

**Laboratory:** Low carnitine, with increase glutaric and 3-OH-glutaric acids in urine. Metabolic abnormalities may be fluctuating and inconsistent. Check for GCDH gene for confirmation.

**Treatment:** glucose – 8-15 mg/kg/min iv, Carnitine – 100 mg/kg/d iv, riboflavine – 100-200 mg/d, lysine restriction – 90-100 mg/kg/d and tryptophan – 20 mg/kg/d, hypoproteic diet – 1,0-1,2 g/kg/d; when with dystonia – diazepam - 0,2- 0,4 mg/kg iv and baclofen- 0,1 mg/kg/dose oral, 8/8 h, increase every 3 days to 0,4 mg, with maximum of 0,8 mg/kg/dose.

All these diseases can be diagnosed and treated in early stage, so we can avoid unnecessary complications in short and medium term.

After the diagnosis, we can offer to the couple, a better genetic counseling and prenatal diagnosis for their future child.

## **Conclusions**

Screening of metabolic diseases in the neonatal period by tandem mass, reduced significantly the morbidity and mortality in children.

All cases need confirmation by enzymatic and/ or gene test.

We can offer to the couple an effective genetic counseling and prenatal diagnosis for their future child.

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