Selective Metabolic Screening from a Neonatology Perspective

Hasan Sinan Uslu, Adil Umut Zübarioğlu, Ali Bülbül

Clinic of Neonatal Intensive Care, Şişli Hamidiye Etfal Training and Research Hospital, İstanbul, Turkey

ABSTRACT

Metabolic diseases are quite rare diseases when considered alone, but they are affecting approximately 1:3.000–4.000 of live births when considering the cumulative meaning. Particularly considering that diseases are inherited genetically, "screening programs for metabolic disease" is very important for those aiming people not only live but also quality to live. Screening feasible in neonates can be analyzed in two groups such as general screening and selective screening tests. The general screening tests are further divided into Newborn Metabolic Screening Program and Expanded Newborn Screening. As a large part of metabolic diseases do not affect the baby at birth, all live-born neonates without any clinical findings are implemented in the process of screening programs at a proper time. This application is called as "Newborn Metabolic Screening Program", and it is made considering the reality of each country with different diseases and limit values. Tandem mass spectrometry (Tandem MS/MS) can not take place in a national screening programme; although a very expensive analytical instrument, the testing is cost-effective because screening of more than 30 diseases is possible with one drop of blood. This practice has been widespread since the last 15 years defined as "Expanded Newborn Screening". Newborns with a positive family history for metabolic disease (defined as metabolic disease or death from a metabolic disease in the family) and/or neonates with clinical symptoms suggestive of metabolic diseases; implementation of selected tests for targeted metabolic disease or metabolic disease group considered as "Selective Metabolic Screening Tests". This is, usually, a strategy carried out in developing countries. The aim is to get a clue to define the maximum number of possible metabolic diseases in the shortest period that can lead to serious problems. On the other hand, it should be known that if the "selective screening test is negative", it does not mean "there is no disease". Newborn metabolic screening tests performed for the early recognition of inherited metabolic diseases is basically a public health service. Considering this aspect, it is not only a laboratory service but also an integration of systems including education, data assessment, diagnosis, treatment, and long-term management approach. *(JAREM 2015; 5: 39-46)*

Keywords: Newborn, selective, metabolic, screening

Metabolic diseases are rare diseases when considered alone; however, considering the cumulative perspective, they affect approximately 1 in 3,000–4,000 live births (1-3). In Turkey, where the rate of consanguineous marriages is higher than that of the world average, considering that diseases are genetically inherited (The lowest rate is 12.8% in western Anatolia and the highest is 35% in southeastern Anatolia. The mean consanguineous marriage rate across Turkey is 21% and 70% of these are within first-degree cousins), the concept of a perinatal center has not yet been adopted, and the awareness and importance of antenatal care and genetic counseling is not given attention to by legislators who make health policies and even by healthcare professionals; "screening programs for metabolic diseases" are important for those who aim for people to not only live but also provide quality living (4, 5). In the report published by the European Agency for Health and Consumers in 2011, the absence of a database in our country among 40 countries is a sad and serious discordance (6). Carlson (7) describes newborn screening programs as the most important development in pediatrics. Newborn metabolic screening testing, which is performed for the early detection of inherited metabolic diseases, is essentially a public health service. Considering this aspect, it is not only a laboratory service but also the integrity of systems including education, data evaluation, diagnosis, treatment, and long-term administrative perception.

Difficulties and Outcomes in the Diagnostic Approach to Metabolic Diseases

Delayed diagnosis: False facts known to be true…

- They are rarely seen.
- Comprehensive knowledge is required for the diagnosis of metabolic diseases, and it is also necessary to know biochemical reactions very well. Therefore, it is very difficult to establish a diagnosis.
- Because patients specific symptoms, the diagnosis should be made after frequently seen diseases, such as sepsis, intracranial hemorrhage, and congenital heart diseases, are ruled out.
- Patients can not be treated even if the diagnosis is established.
- All metabolic diseases result in death or mental retardation.

Delayed diagnosis causes;

- Development of serious permanent sequelae,
- Death of patient before being diagnosed, and
- Disappearance of the chance of prenatal diagnosis in next pregnancies.

Address for Correspondence: Dr. Hasan Sinan Uslu,
Şişli Hamidiye Etfal Eğitim ve Araştırma Hastanesi, Yenidoğan Yoğun Bakım Kliniği, İstanbul, Türkiye
Phone: +90 532 737 00 15 E-mail: sinanuslumd@hotmail.com

Received Date: 27.04.2015 **Accepted Date:** 28.04.2015

DOI: 10.5152/jarem.2015.755

Metabolic Screening Tests in Newborns: Classifications and Definitions

1. General Screening Tests

1. A. Newborn Screening Program

All live-birth newborns without any clinical findings undergo screening programs because no evidence can be seen at birth in most metabolic diseases. In accordance with the "Metabolic Screening Program for Newborns", all newborns (nonselective ones) undergo different screening tests for different diseases considering the realities of each country; also, different reference values are applied. According to the Wilson and Jungler criteria of the World Health Organization, diseases that will be included in national screening programs must have the following features: 1. It must be a significant health problem with a high incidence in the community (more frequent than 1:20,000); 2. The natural course of the pathological condition must be well understood; 3. It must be able to be recognized in its early stages; 4. It must be an appropriate testing procedure; 5. The test must involve the entire population of the society; 6. The repeating intervals of the test must be clearly determined; 7. It must have an efficient treatment; 8. It must have the opportunity of a definite diagnosis; 9. It must be a method that is agreed on which patients will be treated; and 10. The cost–benefit ratio of the test must be economically balanced (8). The metabolic screening criteria of the American College of Medical Genetics cover the scoring method associated with 1. Clinical characteristics (e.g., incidence, burden of disease that is not treated, and phenotype); 2. Analytical characteristics of the screening test (availability); and 3. Diagnosis, treatment, and management of acute and chronic forms. If the score is ≥1200, the test is appropriate for being a screening test. If the score is <1000, it is inappropriate. If the score is between 1000 and 1199, efficient treatment or the natural course of the disease has not yet been revealed and studies are ongoing (9). The development of a country's health system is in parallel with the number of diseases included in this list. In our country, national screening programs are managed for hypothyroidism (temporary 1:1.973, dysgenesis 1:3.707, and dyshormonogenesis 1:9.140), phenylketonuria (1:4.500), biotinidase (1:11.144), and cystic fibrosis (1:3.000).

1. B. Expanded Newborn Screening

The screening test of tandem mass spectrometry (tandem MS/MS), which is not included in the national screening program, is applied because it provides cost-effective testing despite being a very expensive analytical device, and it is possible to screen newborns for over 30 disorders with only one drop of blood. This procedure, which has become more common within the last 15 years, is referred to as the "Expanded Newborn Screening". In the framework of this program, diseases that do not conform to the Wilson and Jungler criteria are also screened, and different screening lists are formed due to various ethical problems (9). The following disorders can be screened through expanded newborn screening: fatty-acid oxidation defects, urea cycle disorders, amino acid metabolism disorders, organic acidemia, galactosemia for some countries, congenital adrenal hyperplasia, some lysosomal storage diseases, sickle-cell anemia, congenital infections, hemoglobinopathies, and alpha-1-antitrypsin deficiency. Many developed countries have expanded their neonatal screening programs along with advances in MS/MS technology. For instance, while Australia includes all diseases that can be screened with MS/MS analysis in expanded newborn screening tests, the number of diseases is 29–57 in the USA, 17 in Holland, 13 in Denmark, 12 in Germany, 5–30 in China, and 5–50 in India. However, Pakistan and Bangladesh includes only congenital hypothyroidism and Iran includes only congenital adrenal hyperplasia in their screening programs (10, 11).

2. Selective Metabolic Screening Tests

The application of tests selected for the targeted metabolic disease or metabolic disease group in newborns with a positive familial history of a metabolic disease and/or with clinical symptoms suggesting a metabolic disease is accepted as "Selective Metabolic Screening Tests". It is a strategy that is generally performed in developing countries. The goal is to obtain clues that are as economical as possible for defining many metabolic diseases earlier (12). In this program, laboratory work is expensive and detailed, and evaluations and consultations for patients are very important for accurate approaches. It requires experience and expertise from this aspect. On the other hand, it must be known that a negative selective screening test result does not mean that there is no disease (13).

Why are Selective Metabolic Screening Tests Important?

Contrary to the approach leading to delayed diagnosis, the prediagnosis of metabolic diseases can be established with familial history, clinical findings, and first-step examinations that can be performed for every patient, and an emergency approach can be taken. At this point, well-planned and accurately interpreted selective metabolic screening tests are critical.

Important Points Related to Selective Metabolic Screening Tests

- Any test alone is insufficient for diagnosing all metabolic diseases.
- If samples for the diagnosis of congenital metabolic diseases are not properly taken at the time when characteristic findings of the disease occur, the specific diagnosis can be missed. A second chance may not be available, particularly for diseases having an acute impairment course.
- The tests to be chosen must be determined according to the clinical findings of the patient and the results of routine biochemical examinations.
- It must be kept in mind that the primary role of selective screening tests is not to establish the final diagnosis, but to screen.
- Although possible metabolic diseases can be detected with routine biochemical tests, which can be applied to all patients, and first emergency treatment can be provided, more specific metabolic tests are required establishing the final diagnosis.
- The negative result of selective screening tests does not mean that there is no disease.

What are Selective Metabolic Screening Tests?

Relying on the above-mentioned important points, selective metabolic screening tests will be categorized, and information

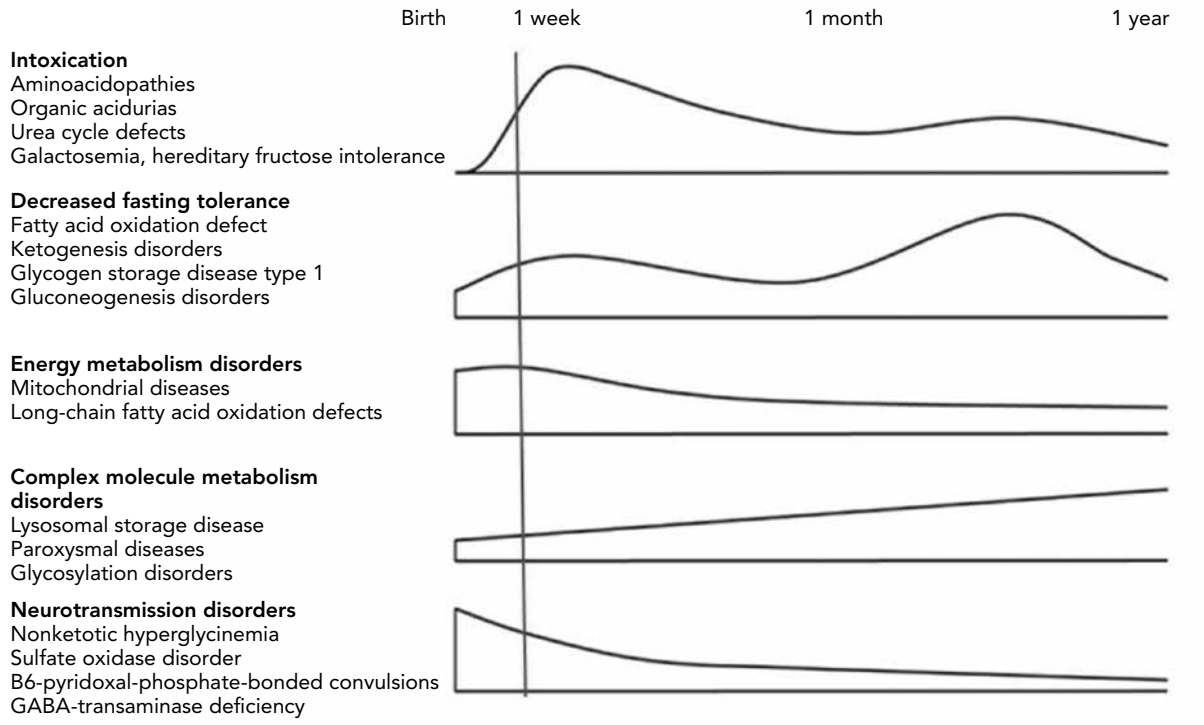

Figure 1. The presentation periods of symptoms in metabolism diseases

on the collection, timing, protection, and transfer conditions of samples will then be given. As in many areas of medicine, knowledge on selective tests for metabolic diseases differs depending on countries, regions, centers, and even clinicians in the same clinic to a great extent because no consensus has been reached on them and they are controversial and didactic.

They are tests chosen for patients having no clinical symptoms, having a suspected familial history (consanguinity between mother and father and concomitant sudden infant death syndrome and/or undiagnosed death history in the neonatal period or sudden infant death syndrome alone and/or undiagnosed death history in the neonatal period), or nonspecific findings. The goal is to define a greater number of problems that can occur in newborns using screening tests at the 48th–72nd hours (red line) (Figure 1).

A. Urine analyses

Metabolic Spot Urine Tests

General considerations for screening tests of spot urine:

1. Patient preparation: It is necessary to determine whether a patient receives any drug or not, he/she has an active infection or not, and he/she has been recently transfused or not.

2. Taking sample: The best sample must be taken before a patient is given any intravenous (IV) fluid. A fresh urine sample must be taken (if possible, in the first 30 min as bed-side monitoring), and the samples that will not use within the first 30 minutes must be frozen and stored.

3. Pre-treatment: Samples sent to the laboratory are examined with regard to factors that can cause false positivity (density, pH, protein, ketone, nitrite, and leucocyte esterase).

Dinitrophenylhydrazine (DNPH) test

Definition: Urinary excretion of alpha-keto acid is evaluated.

Screened diseases: Classical phenylketonuria, maple syrup urine disease, and tyrosinemia types 1 and 2.

False positivity: In patients having ketosis.

Interference: Radio-opaque substances and some antibacterial drugs (high-dose ampicillin).

Working on the sample: Ten drops of DNPH are added to 1-mL filtered urine (100-mg dinitrohenylhydrazine is dissolved in 100-mL HCL and stored in brown bottles in a refrigerator). The formation of yellow or chalk white precipitate in 10 min indicates a positive reaction.

Cyanide nitroprusside test

Definition: Urinary excretion of cysteine and homocysteine is evaluated.

Screened diseases: Homocystinuria and cystinuria.

False positivity: In the use of acetoacetate, penicillamine, captopril, and penicillin.

False negativity: Bacterial contamination.

Taking sample: A fresh urine taken in the morning must be used.

Working on the sample: A few drops of concentrated ammonia and 2 mL of 5% sodium cyanide solution are added to 5-mL urine. There is a 5–10-min wait for the completion of reaction. Then, 5 drops of fresh 5% Na nitroprusside are added to the mixture, and the solution is mixed. Normal urine appears as yellow or shaded

brown. However, if the urine includes sulfur, it appears purple red, and this indicates a positive reaction.

If silver nitrate is used instead of sodium cyanide a purple solution, which is specific to homocystinuria, is seen.

Search of reductant substances in urine

Definition: Urinary excretion of reducing sugars (glucose, galactose, fructose, lactose, mannose, and xylose, but not sucrose) is evaluated. The sugars forming aldehydes or ketones in an alkaline solution are reductants.

Screened diseases: Galactosemia, hereditary fructose intolerance, and tyrosinemia types 1 and 2.

False positivity: Homogentisic acid (alcaptonima) is one of the reductant substances in the urine.

Interference: The use of contrast agent, ascorbic acid, contrast substances , ascorbic acid, cephalosporin, and ampicillin.

Working on sample: Besides Benedict and Fehling reagents, the sample is often operated with Clinitest tablet. Five drops of urine and 10 mL of water are put into a test tube and then mixed. A Clinitest tablet is added, and the tube is mixed after 15 s. The appearance of blue indicates a negative reaction. Yellow-green-tile red indicates a positive reaction. In case of a low amount of glucose in the urine, a red precipitate must be sought after waiting for 15 min.

B. Blood Analysis

Tandem MS/MS – Successive Mass Spectrometry

Historical Perspective
With the identification of isovaleric acidemia (IVA) with gas chromatography mass spectrometry by Tanaka et al. (14) in 1966, the device was used for the diagnosis of metabolic diseases. In the 1990s, Edwin Naylor and other researchers began to use tandem mass spectrometry for measuring blood acylcarnitine and amino acid levels without gas chromatography (15). This resulted in a significant breakthrough in newborn screenings, and many congenital metabolic diseases affecting blood acylcarnitine and amino acid levels could be screened using the same sample in a short time.

Working Principle of Mass Spectrometry
Mass spectrometry works based on analyzing charged particles that move in a magnetic or electrical field by differentiating them from other particles according to the mass/charge ratios (16).

Of blood samples taken on the Guthrie card, the portion obtained through methanol is loaded to the device by the "soft ionization" or "electrospray" method. Ionized and nonionized molecules are separated in the first filtration. Then, ionized main molecules are sent to the "collision chamber" of the device. Here, the main molecules are fragmented into electron-charged smaller particles with the effect of inert gases (e.g., argon gas) (fragmentation). Small ions fragmented from the main molecule are separated based on their masses in the second filtration. Molecules are fragmented into ions according to their mass/charge ratio (17, 18). The main molecules and small ionized molecules, which are a precursor of a specific main molecule, are detected through two mass spectrometries in the device and are then grouped. The spectrum formed by small molecules separated from the main molecule is characteristic of the main molecule. Because the device includes two mass spectrometries, it is referred to as MS/MS (19).

Why Tandem MS/MS?

- It requires a few samples. The collection and storage of samples are easy. It is simple to carry.
- It can screen many diseases at the same time and in a short time (approximately 2–3 min).
- It is cost-effective.
- The rate of false negativity is low (0.26%).

Ideal Timing
In newborn metabolic screening tests, the most appropriate period for the detection of a disease is the 48th–72nd hour after birth. The goal is detect intoxication-type metabolic diseases. Adequate nutrition of newborns and pathological metabolites reaching the level at which they can be analyzed depending on the impairment in the metabolic pathway are considered as the basis. The time of collecting the blood sample can vary in some situations. If fatty acid oxidation defect is selectively targeted, it must be performed in 24–36 h. If a mother is discharged from the hospital early, the sample must be taken from the baby at a time close to discharge. However, in such situations, the test must be repeated by taking the blood sample before the 14th day of life (16, 19, 20).

Special Considerations
The sample must be taken before the procedure for infants who are transfused, given parenteral nutrition (PN) or dialysis, and given corticosteroid, antibiotic, or dopamine treatment.

Because transfusion; causes false negative results for galactosemia and hemoglobinopathies, screening must be repeated 3–4 months after the last transfusion. Other screening test results for inherited diseases have the risk of false negativity within 4–72 h after transfusion.

PN; can lead to false positivity for amino acid and fatty acid examination. The abnormal result in more than one amino acid can develop secondary to increased free amino acid levels due to PN or liver diseases. Because PN fluids include medium-chain fatty acids, they can cause high fatty-acid levels in the blood. Prolonged PN can also induce carnitine consumption and cause false positivity with regard to IVA (high level of C5). In infants for whom abnormal results have been obtained in the screening test, the test must be repeated 24–72 h after ending PN.

Maternal problems; because temporary hypothyroidism [high thyroid stimulating hormone (TSH) levels] will occur in the babies of mothers treated with propylthiouracil due to hyperthyroidism and the effect of drugs will last for 7–14 days in these babies, the test must be repeated in the second week.

On the other hand, in the babies of mothers with congenital adrenal hyperplasia (CAH), 17-hydroxyprogesterone (17-OHP) will result in false positivity, and the test must be performed on the 3rd–7th day after the birth.

Temporary hyperpheylalaninemia can be detected in the babies of mothers with uncontrolled phenylketonuria (PKU). It is waited for this condition to return to normal in 12–24 h provided that the baby does not have PKU.

In newborns whose mothers have taken steroid therapy during their pregnancies, CAH tests can give false positive results. This effect depends on the type and dose of the steroid and can last for approximately 1–2 weeks. The test must be repeated 2 weeks later.

Maternal carnitine or vitamin B12 deficiency can cause false positivity in C0 (carnitine) and C3 (vitamin B12) results. This effect can continue for days. Moreover, carnitine supplementation given to a newborn can create false negativity and false positivity in other acylcarnitine results. In this situation, the test must be repeated 4 days later.

In unhealthy newborns; stress-induced 17-OHP and immunoreactive trypsinogen levels can be elevated. Therefore, false positivity can occur in terms of CAH and cystic fibrosis (CF). Liver diseases and jaundice can lead to false positivity in many conditions (tyrosinemia, homocystinuria, PKU, CF, and biotinidase deficiency).

In preterm infants or infants with low birth weight; high levels of tyrosine and 17-OHP and low levels of biotinidase can frequently occur. False positivity can be encountered in amino acid impairments because of immature liver enzymes. Again, immature hypothalamic/pituitary/thyroid axis [because of insufficient TSH response to low level of thyroxine (T4)] can result in false negativity for hypothyroidism in premature infants. This situation can continue for longer than 1 month. On the other hand, hypothyroidism is generally temporary. In premature infants (<32nd gestational week), the first sample must be taken around the 48th–72nd hours. If hospitalization is longer than 14 days, it must be repeated before discharge from the hospital. If it is longer than 1 month, the test must be re-performed on a new sample taken in the 1st month. It must be also repeated when corrected age term is reached in premature babies.

Dopamine therapy; can cause false negativity for hypothyroidism by inducingTSH suppression.

Steroid therapy; can suppress TSH and cause false negativity with regard to hypothyroidism. It can also create false negativity in terms of CAH. This effect disappears 1–2 weeks after the end of therapy.

In antibiotic therapy; antibiotics conjugated with pivalic acid (in the production of ampicillin, amoxicillin, cefazolin, and many semisynthetic antibiotics) increase the C5 level (false positivity for IVA). This effect ends 24 h after the discontinuation of the drug.

Early sampling; can cause false positivity for hypothyroidism and CAH because hormone levels fluctuate after delivery. In samples taken within the first 24 h, false positivity can be observed in terms of amino acid and organic acid impairments.

Late sampling; can lead to false negativity in blood samples taken after 48 h from well-nourished infants because fatty acid oxidation defect can be identified only during catabolism. Therefore, if the result of sample is positive in the first sample but negative in the next sample, the first evaluation should not be interpreted as false positivity, and it should be confirmed with diagnostic tests. It is recommended that the sample for tandem MS/MS should be taken between the 24th and 36th hours, if possible. False positivity for fatty acid oxidation defects is a rare situation.

On the other hand, the results of screening tests performed after the occurrence of symptoms in diseases that can appear with early metabolic crisis will be completely impaired, and the tests will lose their significance.

In patients undergoing dialysis, the tests must be repeated on the 6th, 30th, and 60th days.

In addition, nutrition without protein causes false negativity (16, 19-21).

Taking Proper Blood Samples

The most appropriate region for drawing blood in newborns is the heel. The heel is slightly warmed and is held below the heart level. The heel is divided into 3 equal longitudinal areas with imaginary lines and is cleaned and dried. One of the lateral medial parts of the heel is punctured with a lancet. The goal is to take 1 big drop of blood through a one-time punctuation process without squeezing the heel (inaccurate result can be obtained due to leaking tissue fluids). The first drop of blood is wiped off (for the blood not to be contaminated with alcohol), and the second drop of blood is absorbed in the marked circle on a prepared special paper (Guthrie card). The heel must never be touched with the paper, and only the drop of blood must be absorbed in the paper.

- Venous blood sample or samples taken into the hematocrit tube (because the blood mostly accumulates at the point where the blood sample is first touched and blood with increased viscosity does not spread from that point) should not be used because the examination will be performed by separating 1–4 µl of blood from a 3–3.5 mm-diameter disc in the related laboratory.
- The tissue should not be squeezed too much while drawing blood (due to leaking tissue fluids).
- Any tube containing EDTA should not be used.
- Areas where the blood sample will be absorbed should not be touched by hand.
- It should be kept in mind that the volume of absorbed blood, hematocrit level of absorbed blood, and duration of blood absorption are variables that affect measurement.
- Blood absorption should not be performed from the same points in the circles on the filter paper.
- It should not be applied on both sides of the paper.
- There should be no scratches on and damages to the filter paper.
- The card should not be exposed to heat, moisture, and direct sunlight.
- There should be no contamination from external factors such as urine, stool, and milk.

- After drawing blood, introductory information about the newborn baby should be legibly written on the form. Personal identifying information, the names of drugs that are used, the state of nutrition, and the state of transfusion should be specified.
- After blood samples are horizontally dried at room temperature, they should be rapidly sent to the center where they will be analyzed.
- The samples should reach to the laboratory before 72 h, and should not be waited for collecting other babies' samples.

Causes of False Positivity

- Low cut-off levels
- Premature (CAH and amino acid metabolism disorders) and sick newborns
- Feeding with total PN
- Vitamin C and vitamin B12 deficiencies.
- Early sampling
- Protein loading (in those consuming cow's milk).
- Specific drug therapies (carnitine, valproic acid, pivalic acid, benzoic acid, and medium-chain triglycerides).
- Some maternal problems (those who receive propylthiouracil, steroids, and B12 therapy for hyperthyroidism, CAH, and uncontrolled PKU).
- Errors related to sampling [contaminated sample, collecting samples in the first 24 hours, sample collection technique (taking a thick drop), drying technique (e.g., drying in an oven), storing and transport method, and laboratory error].

Causes of False Negativity

- High cut-off levels
- Slight clinical forms
- Prematurity (temporary hypothyroidism)
- Innutrition or low-protein diet
- Sampling (contamination, insufficient sample, and denaturation due to the effect of heat and alcohol)
- Transfusion (in galactosemia and hemoglobinopathies)
- Late sampling (fatty acid oxidation defect)

Professional and Legal Liability

Conditions that can affect the results of newborn screening tests include technical problems and the lack of communication among the laboratory members, family, and clinician. In some diseases, some prerequisites are needed for optimal test results to be obtained. Unless provided, the sampling becomes inaccurate, and the repetition of sample collection procedure is required. It is the physician's responsibility to inform the family about the procedure, to receive written/verbal informed consents, to collect blood sample in a technically appropriate manner, to keep the sample under suitable conditions, to transport all samples to the related laboratory at the proper time, and to organize and control all these processes. On the other hand, the duty of the institution's administration is to establish and inspect the system.

While receiving written and verbal informed consents of parents before the application of screening tests, there are some important considerations. Firstly, it must be emphasized that screening tests are not diagnostic instruments; therefore, unless the result of the screening test is confirmed with diagnostic tests, some negative psychological problems can develop. In addition, it must be stated that parentsmust accept further examinations in case of any negative result.

Which Diseases are Screened with Tandem MS/MS?

Primarily, most amino acids and acylcarnitines including carbon from C2 to C18 and carnitine (C0) can be detected with tandem MS/MS. With this technique, approximately 45 hereditary metabolic diseases, which are given below, can be diagnosed from the blood sample taken on the Guthrie card from the heels of newborns for "expanded newborn screening" or "selective metabolic screening". The relationship between acylcarnitine and amino acid profiles and metabolic diseases is presented in Table 1. These diseases, which occur not only in newborns but also in any age group, can be screened using tandem MS/MS. It has been demonstrated that lysosomal storage diseases can also be screened with recently developed techniques (22).

- Amino acid metabolism diseases
  - Phenylketonuria
  - Maple syrup urine disease
  - Benign hyperphenylalaninemia
  - Homocystinuria (cystathionine ß-synthase deficiency)
  - Tyrosinemias (succinylacetone for type I)
  - Tyrosinemia type II
  - Biopterine cofactor biosynthesis defect
  - Tyrosinemia type III
  - Biopterine cofactor regeneration defect
  - Hypermethioninemia (methionine adenosyl transferase deficiency)
- Fatty acid oxidation impairments
  - Medium-chain acyl-CoA dehydrogenase deficiency
  - Very long-chain acyl-CoA dehydrogenase deficiency)
  - Long chain 3-hydroxy acyl-CoA dehydrogenase deficiency
  - Trifunctional protein deficiency
  - Carnitine uptake defect
  - Short-chain acyl-CoA dehydrogenase deficiency
  - Medium/short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency
  - Medium-chain ketoacyl-CoA thiolase deficiency
  - Carnitine palmitoyltransferase II deficiency
  - Carnitine/acylcarnitine translocase deficiency
  - Carnitine palmitoyltransferase I deficiency-liver
  - Dienoyl-CoA reductase deficiency
  - Carnitine palmitoyltransferase I deficiency-muscle
- Organic acidemias
  - Biotinidase deficiency
  - IVA
  - Glutaric aciduria type I
  - 3-Hydroxy-3-methylglutaric aciduria
  - Methylcarboxylase deficiency

Table 1. The relationship between acylcarnitine and amino acid profiles and metabolic diseases

	Abbreviation	Change	Disease
A)Acylcarnitines			
A.1.Organic acidemias			
Propionyl carnitine	C3	High (↑↑)	Propionic acidemia, methyl malonic acidemia
Propionyl carnitine	C5	(↑↑)	Isovaleryl acidemia, 2-methyl butyryl CoA dehydrogenase deficiency
3-hydroxy isovaleryl carnitine	C5OH	(↑↑)	3 ketothiolase deficiency, 3-hydroxy 3-methyl glutaryl CoA lyase deficiency, 3-methyl crotonyl CoA carboxylase deficiency
Glutaryl carnitine	C5DC	(↑)	Glutaricacidemia type I
A.2.Fatty acid oxidation disorders			
Butyryl carnitine	C4	(↑↑)	Short-chain acyl CoA dehydrogenase deficiency, isobutyryl CoA dehydrogenase deficiency
Octanoyl carnitine	C8	(↑↑)	Medium-chain acyl CoA dehydrogenase deficiency, multiple acyl CoA dehydrogenase deficiency
Tetradecanoyl carnitine	C14: 1	(↑)	Very long-chain acyl CoA dehydrogenase deficiency
Palmitoyl carnitine	C16	(↑)	Carnitine palmitoyl transferase II deficiency, carnitine acyl carnitine translocase deficiency, multiple acyl CoA dehydrogenase deficiency
Hydroxypalmitoyl carnitine	C16: OH	(↑)	Long-chain hydroxy acyl CoA dehydrogenase deficiency
Carnitine (low)	C (total)	Low (↓↓)	Carnitine transport disorder
B)Amino acid metabolism disorders			
Glycine		(↑)	Nonketotic glycinemia
Valine		(↑)	MSUD
Leucine/isoleucine		(↑)	MSUD
Phenylalanine		(↑)	PKU
Tyrosine		(↑)	Tyrosinemia
Methionine		(↑)	Homocysteinemia and other hypermethioninemia
Arginine		(↑)	Argininemia
Citrulline		(↑)	Citrullinemia, Argininosuccinyl-CoA-lyase (ASAL) deficiency
Argininosuccinic acid		(↑)	ASAL deficiency

C: Carnitine, C3, C5, C5DC, C4, C8, C14:1, C16, C16:OH: aliphatic carnitine esters, referred according to the binding number of monocarboxylic acids to carbon atom with long chain.
MSUD: Maple syrup urine disease ; PKU: Phenylketonuria

- Methylmalonic acidemia (mutase deficiency)
- 3-Methylcrotonyl-CoA carboxylase deficiency
- Methylmalonic acidemia (Cbl A,B)
- Propionic acidemia
- Beta- ketothiolasedeficiency
- Glutaric acidemia type II
- Methylmalonic acidemia (Cbl C,D)
- Malonic acidemia
- Isobutyryl-CoA dehydrogenase deficiency
- 2-Methyl-3-hydroxybutyric aciduria
- 3-Methylglutaconic aciduria
- 2-Methylbutyryl-CoA dehydrogenase deficiency

- Urea cycle defects
  - Citrullinemia type I
  - Citrullinemia type II
  - Argininemia
  - Arginosuccinic acidemia
  - Ornithine transcarbamylase deficiency

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - H.S.U., U.Z., A.B.; Design - H.S.U., U.Z., A.B.; Supervision - H.S.U., U.Z., A.B.; Resource - H.S.U., U.Z., A.B.; Materials - H.S.U., U.Z., A.B.; Data Collection and/or Processing H.S.U., U.Z., A.B.; Analysis and/or Interpretation - H.S.U., U.Z., A.B.; Literature Review - H.S.U., U.Z., A.B.; Writer - H.S.U.; Critical Review - H.S.U., U.Z., A.B.; Other - H.S.U., U.Z.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

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