EFFECTING FACTORS ON THE NEWBORN SCREENING

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Conditions of Specimen Collection

➢ Timing

- The timing of blood sampling is important parameter for accurately interpreting test results for newborn screening.
- The Clinical and Laboratory Standards Institute (CLSI): Recommends blood spot collection on filter paper for screening after 24 h of age preferably between 24 and 48 h of age[1]. (24-72 h)
- The second specimen must be collected between 10 and 14 days of age.

Early sampling (before 24 h):

The infant's biochemical transition from a mother-dependent to an autonomous state

Late sampling (after 48 h):

Delay diagnosis and initiation of treatment for some infants or different patterns of metabolites

Timing of blood sampling

- ✓ **Different patterns were found for different metabolites according to sample timing**
- ✓ **Different cutoff values depending on the infant's age (in hours)**
- ✓ **False-positive / negative screens for some disorders**

Phenylalanine levels decreased sharply in the first 36 h, with very small changes after 48 h

C16OH decreased steadily from 18 to 72 h after birth.

Leucine-isoleucine levels decreased during the first 30 h and then increased.

Tyrosine levels were higher at standard sampling time (24–48 h) compared to late sampling
Free carnitine (C0), its level decreased during the first 48 h.

The early-collection had elevated marker levels for PKU, IVA, MMA ,and CTD.

Conditions of Specimen Collection

➢ Normal feeding

- Normal feeding on breast or formula is required to reveal screening results.

- Protein intake has been adequate to reveal a positive screen, particularly for amino acid disorders

- If protein intake is deemed suboptimal (poor feeding) a further sample should be taken.

Conditions of Specimen Collection

- The patient should preferably be fast.
- Non-acute samples: Pre-prandial in newborns (immediately before the next feeding)
  Ideally collected after at least 3-4 hours fast (e.g. early morning)
- Samples collected post-prandially may show increases in amino acids.
- Soy formula or lack of milk feeding will affect screening for galactosemia.

Infant conditions affecting the newborn screening results

➢ Preterm birth & Gestational age

- ✓ In premature neonates, the false positive rate is higher than the term
- ✓ Various medical therapies, the preterm infant's metabolic system needs, fetal stress, liver, kidney, and adrenal immaturity
- ✓ False-positive results for some conditions (congenital hypothyroidism, congenital adrenal hyperplasia, and phenylketonuria)
- ✓ False-negative results for Lysosomal Storage Disorders

Infant conditions affecting the newborn screening results

➢ Birth weight

- ✓ False-positive results are inversely correlated with gestational age and birth weight, with the exception of increased false-positive results for infants >42 weeks' gestational age.

- ✓ False-positive are 13-fold higher in VLBW infants (at least 1 in 10 VLBW infants had a false-positive result)

- ✓ These babies will require a second sample at a time when their systems are fully functional

Infant conditions affecting the newborn screening results

➢TPN

- Preterm or LBW neonatal: Need high protein supplementation via total parenteral nutrition (TPN) to prevent catabolism.

- TPN or protein supplementation leads to higher-than-normal amino acid levels in the blood; thus, falsely elevated amino acid profile, fatty acid oxidation, and organic acid disorders and may cause False-positive screening results

- Carnitine supplementation:

  Elevations of acylcarnitines; can mask carnitine transport disorders

- If the baby was admitted to NICU: Another specimen is requested when the infant has been off TPN for 4-24 hours, or on day 14 of life.

- Replacing TPN with a D10W* infusion for 3-4h before specimen collecting, reduced False-positive screening results

- Screening specimens must be repeated after discontinuing carnitine supplementation

- The samples should not be taken from the line that is used to deliver the nutrition or drug.

* (Dextrose 10% in Water)

Infant conditions affecting the newborn screening results

- **Blood transfusions:**
  - Donor cells may cause normal levels of analytes and may result in false normal screening results.
  - Invalidate screening for classical galactosemia, biotinidase deficiency, and hemoglobinopathies
  - Specimen collection immediately after transfusion will affect all newborn screening results (as long as 120 days) and delay diagnosis and treatment.
  - The specimen should be collected prior to transfer, regardless of infant's age.
  - If a post-transfusion specimen is necessary: 48 hours post-transfusion and 90 days after the final transfusion.

Infant conditions affecting the newborn screening results

- **Medical therapy:**
  - Some antibiotics (such as ampicillin, pirampicillin, and cefotaxime) that are given to mothers during labor or to newborns:

Can induce an increase in the level of acyl-carnitines (C5, C14:1, and C16:1-OH)

Maternal conditions affecting the newborn screening results

- **Maternal B_{12} deficiency**

The altered profile of C3 (propionyl-carnitine), the C3/C2 (C3/acetyl-carnitine) ratio

C16: 1OH\C17 (3-hydroxy-hexadecenoylcarnitine) and methionine is attributable to maternal vitamin B_{12} deficiencies

(70% of the Methylmalonic acid : False Positive result)

Maternal conditions affecting the newborn screening results

- **Maternal PKU, and 3-MCC deficiencies**

Transient elevation of phenylalanine (first 24 h)

Elevated 3-hydroxyisovalerylcarnitine (C5-OH) on a neonatal screening test

- **Maternal carnitine deficiency**

Low levels of free carnitine (C0)

Maternal conditions affecting the newborn screening results

➢Complication of pregnancy:

- Preeclampsia:

Alanine (ALA), free carnitine (C0), acetylcarnitine (C2), octenoylcarnitine (C8:1) and linoleoylcarnitine (C18:2) were significantly elevated in infants born to preeclamptic mothers

➢Mother lifestyle:

- Active mothers during pregnancy: increase the levels of glutamine (GLN) and glutamate (GLU) in the metabolic profile of newborns
- Smoking during pregnancy: acylcarnitines, such as C18: 1, C18: 2, and C14: 1, were all higher in the metabolic profiling of newborns

Maternal conditions affecting the newborn screening results

- Mothers heterozygous for a FAOD and pregnant with an affected fetus may develop severe preeclampsia, Fatty liver of pregnancy, and the HELLP syndrome, and may deliver a premature, intra-uterine growth-restricted (IUGR) infant.

- ✓ Possible for mitochondrial fatty acid oxidation deficiency

HELLP syndrome (hemolysis, elevated liver enzymes, low platelets)

Filter Paper Collection Form

- ✓Birth history and effecting factors on specimen collection forms should be complete
- ✓The feeding type box should be clearly marked for “Breast”, “Formula”, “soy”, “TPN” or “NPO” (and those not yet receiving milk)
- ✓The “Antibiotic” check box on the specimen collection card should be marked
- ✓ Maternal diseases

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for your
attention!