Nutrition Support and Newborn Screening in the NICU Population: Is There A Link?

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ABSTRACT

Background: Recent research is revealing the high rate of false-positive screening results for IEMs in the NICU population. No study published to date has specifically studied the possible relationship between nutrition and newborn screening in this population.

Objective: It is suspected that NICU infants who receive PN are more likely to have abnormal newborn screening results than infants who receive EN. An understanding of the role of nutrition will assist in developing protocols for screening in the NICU and decrease false-positives.

Design: Infants admitted to the NICU between January 1-June 30, 2009 were included in this retrospective chart review study (n=339). The type of nutrition and timing of its initiation was recorded and compared to newborn screening results to identify correlations with false-positives. Statistical analysis included means, percentages, Fisher’s exact test, Chi-square test, and the Cochran-Mantel-Haenszel test.

Results: Nutrition type was significantly associated with newborn screening (p<0.001); those who received parenteral nutrition were more likely to have a false-positive. For infants who also received PN, EN of breast milk exclusively increased risk of an abnormal screen more than formula exclusively or breast milk plus formula. The timing of parenteral nutrition had no effect on screening. Premature infants who received PN exclusively had a higher percentage of false-positives than those who received EN.

Conclusions: Although the hypothesis could not be statistically supported, PN appears to contribute to false-positive newborn screens. More research is needed to ascertain the role of EN and GA in newborn screening and to develop standardized protocols.
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Dr. William Wilson has been the driving force behind this project. As the Pediatric Medical Geneticist at UVA, it was he and Barb’s query of the role of nutrition in newborn screening that sparked the idea for this investigation. He provided guidance to me throughout the design and data collection phases of my research which added a degree of quality to my study that would not have been present without his help.

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Finally, I’d like to thank my colleagues Dena Goldberg and Monica McAllister. These clinicians provide nutritional care to the infants in the NICU at Roanoke Memorial and were very helpful to me as I was learning the ins-and-outs of this population.
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Chapter 1: Introduction

Introduction and Background Information

Medicine and nutrition have both been studied intensely throughout time and intersect in many ways. Throughout the centuries food has been used to restore health to those that were sick. More recently, the potential negative effects of diet have been investigated and much has been learned in the science of medical nutrition therapy. There is no greater example of the balance between the healthy and adverse effects of food as that surrounding Inborn Errors of Metabolism (IEM), in which proper, specialized nutrition is essential to normal growth and development. Unfortunately, there are challenges of diagnosing these disorders in the premature and critically ill newborn population due to factors such as specialized medical care, nutritional support, and the current lack of understanding into their unique metabolic processes. Furthermore, the actual screening process used to detect these conditions has inherent limitations. Therefore, there is a need for more in-depth comprehension of these factors to delineate the potential association between nutrition support and the newborn screen in the preterm and critically ill neonate. Such is the basis of the current research study.

Inborn Errors of Metabolism, sometimes referred to as Inherited Metabolic Disorders (IMDs), are genetic defects that alter the processing of certain nutrients within the affected individual. Usually macronutrient metabolism is affected. Over 200 of these conditions are known and most are passed from generation to generation in an autosomal recessive pattern (1, 2). This mode of inheritance requires that an individual receive two copies of the defective gene (one from each parent) in order for the condition to be present (3). Each disorder affects a specific biochemical pathway or pathways within the body with a wide variety of outcomes.
ranging from no clinical effect to death. Fortunately, many of these conditions can be successfully treated; a significant proportion of these are managed through appropriate nutrition intervention. Knowledge of the specific metabolic pathways involved in each disorder is critical to understanding the nutrition therapy.

There are several different types of IEMs that can be classified according to the general metabolic defect, typically those involving amino and fatty acid metabolism, hemoglobin, and a few various conditions grouped together as “other disorders.” Table 1 lists many of the known types of IEMs and the specific conditions included in each category. A brief overview of these is warranted. For example, amino acid metabolism disorders occur when a specific enzyme is deficient. The lack of that enzyme’s activity leads to an accumulation of a particular substrate that cannot be processed, usually causing organ damage. Often this disordered pathway blocks the normal production of important downstream products, resulting in a deficiency of other metabolites. Phenylketonuria (PKU) is an example of an amino acid disorder in which a deficiency of phenylalanine hydroxylase blocks the conversion of phenylalanine to tyrosine. The nutritional treatment requires restricting the intake of the offending amino acid, in this case phenylalanine. This involves strict adherence to a low-protein diet and supplementation of all other amino acids to ensure adequate nutritional status.

Organic acidemias, such as isovaleric acidemia, are a type of metabolic disorder in which organic acids accumulate and alter the body’s acid-base balance, thereby affecting intermediary metabolism. These conditions are also the result of disordered amino acid processing. Patients with such a condition often experience episodes of metabolic crises such as ketoacidosis, hyperammonemia, seizures, and acidosis, which require immediate treatment to prevent negative outcomes (2, 4).
Some IEMs affect enzymes within the fatty acid oxidative pathway and are thus appropriately dubbed “fatty acid oxidation disorders” (FAO disorders). Implicitly, this group of disorders impacts the metabolism of both ingested and endogenously stored fat. One example of a FAO disorder is Medium-Chain Acyl-CoA Dehydrogenase Deficiency (MCADD). In this condition, defective mitochondrial beta-oxidation can cause hypoglycemia during periods of fasting because fat cannot be metabolized to ketones in the absence of adequate blood glucose. This is of particular concern during infancy and is likely the culprit in many sudden infant death syndrome (SIDS) cases. Preventing death is clearly the primary advantage of early detection (5).

Galactosemia is a type of carbohydrate metabolism disorder in which galactose is improperly metabolized secondary to a deficiency of one of 3 specific enzymes (2, 5). If untreated, the classic version of this condition, which affects the galactose 1-phosphate uridyltransferase enzyme, can quickly become fatal. Other unfortunate manifestations of delayed treatment for galactosemia affect the kidneys, gastrointestinal system, and vision/eye health. Similar to PKU, treatment involves restriction of all dietary galactose (5). A brief description of many specific IEMs can be found in Balk’s recommendations for newborn screening policy change in neonatal intensive care units (NICUs) and is found in Appendix 1 (6). Additional detailed information is given for selected conditions by Levy and Albers (7), whereas Kaye describes the newborn screening process in depth (5).

Population-based newborn screening (NBS) has been established as a preventive public health measure available to all neonates. If infants with specific metabolic disorders are identified by NBS in the first few days of life, treatment can begin and thus neurological and/or physical damage can be prevented. Screening the NICU population presents unique challenges that are beginning to be addressed. Such difficulties are manifest because of the characteristics
of the patients. These infants are often born prematurely (before 37 weeks gestation) and therefore do not have fully developed organ systems, making them automatically at high risk for nutritional and other complications. Gestational age and birth weight can be used to stratify newborns into risk categories. Low birth weight infants weigh less than 2500g, very low birth weight are less than 1500g, and those weighing less than 1000g are classified as extremely low birth weight (8).

Table 1: Types of Inborn Errors of Metabolism

<table>
<thead>
<tr>
<th>Organic Acidemias</th>
<th>Amino Acidemias</th>
<th>Hemoglobinopathies</th>
<th>Other Disorders</th>
<th>Fatty Acid Oxidation Disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isovaleric Acidemia (IVA)</td>
<td>Phenylketonuria (PKU)</td>
<td>Sickle Cell Anemia (Hb SS)</td>
<td>Congenital Hypothyroidism (CH)</td>
<td>Medium-Chain Acyl-CoA Dehydrogenase Deficiency (MCADD)</td>
</tr>
<tr>
<td>Glutaric Aciduria Type 1 (GA-1)</td>
<td>Maple Syrup Urine Disease (MSUD)</td>
<td>Hemoglobin S-beta-Thalassemia (Hb-SβTh)</td>
<td>Biotinidase Deficiency (BIOT)</td>
<td>Long-Chain L-3-Hydroxyl Acyl-CoA Dehydrogenase Deficiency (LCHADD)</td>
</tr>
<tr>
<td>Multiple Carboxylase Deficiency</td>
<td>Homocystinuria (HCY)</td>
<td>Hemoglobin SC Disease (Hb S/C)</td>
<td>Galactosemia</td>
<td>Very Long-Chain Acyl-CoA Dehydrogenase deficiency (VLCADD)</td>
</tr>
<tr>
<td>Propionic Acidemia (PA)</td>
<td>Tyrosinemia Type 1 (TYR-1)</td>
<td></td>
<td>Cystic Fibrosis (CF)</td>
<td>Carnitine Uptake Defect (CUD)</td>
</tr>
<tr>
<td>MethylMalonic Acidemia</td>
<td>Arginosuccinic Acidemia (ASA)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table derived from Goodin (2) and Balk (6)

Often the NBS results for a critically ill or preterm infant are abnormal even when no disorder is present. Such a finding is a false-positive and may be the result of medication, nutrition, or the unique metabolic processes in this population (9). Researchers are now working to ascertain the exact relationships into these potential causative factors of abnormal screens in this population.
Epidemiological Information

Overall, IEMs occur once out of every 4,000 live births in the United States. It is interesting to note that up to 20% of term infants who develop sepsis of unknown origin may have an IEM (10). The National Institutes of Health has published the frequencies of specific conditions through the U.S. National Library of Medicine website. They report that the incidence of PKU in America is about 1 in every 10,000-15,000 newborns. Classic galactosemia (Type I) is more common than type II, affecting 1 in 30,000-60,000 newborns versus 1 in 100,000 respectively; type III is even more rare. About 1 in every 17,000 people in the U.S. has MCADD while 1 in 250,000 is affected by isovaleric acidemia (11). While these overall numbers provide much information, it is important to assess smaller subsets of epidemiological information for IEMs.

Some genetic conditions affect certain populations more frequently than others. For example, while only 1 in 185,000 infants throughout the world has MSUD, 1 out of every 380 newborns in the Old Order Mennonite population is affected. Similarly, homocysteinuria is most common in Ireland, Germany, Norway, and Qatar but overall, the incidence is 1 in every 200,000 to 335,000 people (11). A careful examination of regional data is essential to understanding the setting for the proposed research.

In Virginia, there is a birth registry of children under age 2 with congenital anomalies referred to as VaCARES (Virginia Congenital Anomalies Reporting and Education System). It contains the state’s epidemiological information and is managed by Virginia Commonwealth University (VCU). The state currently screens for 28 disorders, closely reflecting the panel recommended by the American College of Medical Genetics (12).
VaCARES reports there are about 117 cases of metabolic and endocrine disorders diagnosed each year per 10,000 live births. Specifically for PKU, about 1 case per 10,000 live births is diagnosed annually. Although this is the most recently available data, the information is somewhat dated; these numbers come from the 1989-1998 averages. In the nearly 47 years that Virginia has been screening for PKU, a total of 150 cases have been confirmed. In that same time frame, Alabama has diagnosed 113 cases while Oregon has found 18 (13). It is important to note that these numbers are not standardized for population differences.

Patients in the Commonwealth with an IEM are currently cared for by 1 of 3 treatment centers depending on geographic location: Eastern Virginia Medical School, the University of Virginia, or the Medical College of Virginia through VCU. Table 2 shows the types and incidence of some IEMs diagnosed in Virginia to date in 2009 as well as since screening began (12). It must be noted that the amount of time each condition has been screened varies. As new technology has emerged, Virginia has expanded its newborn screening program, thus, a lower incidence rate reported here does not necessarily mean a lower overall incidence rate.

Table 2: Incidence of IEMs in Virginia

<table>
<thead>
<tr>
<th>Condition</th>
<th>Diagnoses in 2009 (as of 7/15)</th>
<th>Diagnoses Since Screening Began</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotinidase Deficiency</td>
<td>2</td>
<td>34</td>
</tr>
<tr>
<td>Carnitine Uptake Defect</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Citrullinemia</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Classic Galactosemia</td>
<td>1</td>
<td>106</td>
</tr>
<tr>
<td>Galactosemia Variant</td>
<td>9</td>
<td>126</td>
</tr>
<tr>
<td>Glutaric Aciduria Type 1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Isovaleric Acidemia</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Long-Chain 3-OH Acyl-CoA Dehydrogenase Deficiency</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Medium-Chain Acyl-CoA Dehydrogenase Deficiency</td>
<td>1</td>
<td>35</td>
</tr>
<tr>
<td>Maple Syrup Urine Disease</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Phenylketonuria</td>
<td>1</td>
<td>151</td>
</tr>
<tr>
<td>Propionic Acidemia</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Tyrosinemia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Very Long-Chain Acyl-CoA Dehydrogenase Deficiency</td>
<td>0</td>
<td>8</td>
</tr>
</tbody>
</table>

Table derived from the National Newborn Screening Information System (13)
The expanding knowledge of IEMs has been as beneficial for the patients as it has been puzzling for medical care practitioners. While morbidity and mortality from these conditions has decreased sharply since research began, many questions remain to be answered. Specifically, the screening and diagnostic processes need to be refined to maximize diagnoses and minimize false-positives. The potential role of nutrition support in the NICU population must be understood in order to develop the most appropriate screening protocols and reduce added cost.
References

Chapter 2: Literature Review

History and Background of Newborn Screening

Most genetic metabolic conditions result in significant health impairments when left untreated. For example, untreated maple syrup urine disease (MSUD) quickly leads to severe neurological impairment and/or death (1). Another illustration is that infants with phenylketonuria (PKU) who are not started on the special diet soon after birth develop mental retardation as a result of phenylalanine (phe) accumulation. Clearly, it is best to identify and treat each infant with an inborn error of metabolism (IEM) before symptoms and irreversible damage occur (2). The newborn screening system has been the avenue for early diagnosis and treatment resulting in normal development of affected children, often by utilizing diet modification (1). Newborn screening (NBS) is a pro-active and critical step in detecting certain genetic conditions before disability or death develop (3). It is the sole intent of this program to reduce the morbidity and mortality of genetic and metabolic diseases before symptoms even occur (4).

As the name implies, the newborn screening program aims to detect inherited metabolic disorders (IMDs) very soon after birth to avoid the aforementioned negative outcomes. It was first introduced in 1962 in Massachusetts as an initiative by Robert Guthrie and Robert MacCready to detect and prevent complications of PKU. They used a bacterial assay for phenylalanine that quickly became known as the “Guthrie test” (5). A capillary blood sample for the “Guthrie specimen” is usually obtained from a heel prick but sometimes is taken from a venipuncture or venous or arterial catheter. The results of the screen are the same regardless of the source of the specimen (1). Filter paper specimen cards are used to collect several blood
spots which are then allowed to dry and are sent to a lab for analysis (6). Most states and some European countries were screening for PKU by the start of the 1970s. Levy and Albers summarize the success of PKU screening in their NBS overview by stating that “newborn screening has virtually eliminated mental retardation from PKU” (1).

Since the initial NBS program, many more assays have been developed to detect several other genetic disorders, including galactosemia, MSUD, congenital hypothyroidism, biotinidase deficiency, and sickle-cell anemia (1). Each aims to detect an abnormal concentration of the metabolite affected in the disorder. Genetic screening can now detect over 20 types of inherited conditions (7).

The NBS program is not simply a genetic test at birth but rather an entire system of diagnosis, treatment, education, follow-up, crisis management, and evaluation (3). There are 3 possible results of the newborn screen: normal, abnormal, and critical. All enzymes are present and there are no elevations of any metabolic analyte in a normal result. Abnormal means there are mild elevations of one or more analytes while a critical result requires immediate follow-up. If a screen is abnormal, the child’s primary care physician is notified as well as the institution from which the sample was collected. A repeat screen should then be completed. If the results are again abnormal, the child and family should be referred to a metabolic treatment center for further workup and diagnosis. If the NBS results are critical, they are typically reported directly to a metabolic treatment center for an urgent intervention (6). Diagnostic testing should specifically assess metabolites involved in the suspected disorder rather than simply repeating the screen (8). An accurate diagnostic workup is critical for optimal outcomes and to establish the mode of therapy (9).
Despite improvements in diagnostic technologies, false-positive screening results still happen. False-positives occur when a newborn screen shows the presence of a metabolic abnormality that suggests a genetic disorder when in fact that disorder is not present. This is a problem because a precise and timely diagnosis is necessary to establish the mode of therapy. Most of the time, false-positives are transient findings as opposed to lab error (7). These transient findings may be caused by biochemical or hormonal fluxes (10).

Waisbren et al. conducted a prospective study in which 2 groups of children with an IEM born in New England were compared by their mode of disease identification (7). The first group (n=50) was found to have an IEM via NBS soon after birth. In the second group (n=33), each child was diagnosed with an IEM at the onset of the clinical symptoms that surrounded the metabolic crisis including vomiting, dehydration, and hypoglycemia. The researchers conducted neurodevelopmental and medical evaluations of each patient, along with interviews of the parents. The children were evaluated using 3 separate tests: the 2nd edition Bayley Scales of Infant Development to assess for developmental delays, the 4th edition Stanford-Binet Intelligence Scale to measure mental capacity, and the Vineland Adaptive Behavior Scales to measure parental perceptions of the child’s communication and motor skills.

In this study, 60% more children in the clinically diagnosed group either endured symptoms at diagnosis or complications after diagnosis than those diagnosed at birth via NBS. Furthermore, children in the clinical group were 3 times more likely to need some type of special intervention when compared to those identified by NBS. Such interventions included home nursing care and other special services. Over half of the children in the clinically-identified group were hospitalized before age 6 months compared to about a quarter of those in the other group. Additionally, almost 50% of the children diagnosed clinically had some sort of deficit
measured by the Vineland Adaptive Behavior Scale. Such deficits included those in communication, socialization, and motor skills. No child in the NBS group was noted to have any deficit (7). Clearly, there are improved health outcomes related to early identification of these IEMs.

This study also measured stress as perceived by the parents around the time of diagnosis. Mothers of children identified clinically experienced significantly more stress than mothers of children identified by NBS (p < 0.001). This was measured using the Parenting Stress Index (PSI) tool. Parents of the NBS group not only experienced less stress, but they were happier with their support network than parents in the clinical group. Overall, the researchers found that children with metabolic disorders who were diagnosed soon after birth using NBS are less likely to experience developmental and health problems than those diagnosed clinically (7).

An additional tier of information in this study came from assessing stress levels in mothers of infants with a false-positive initial NBS (n=94) as compared to mothers whose infants had normal screens (n=81). Mothers in the false-positive group scored significantly higher on the PSI tool than those of the infants with normal newborn screens (p < 0.001). A manifestation of this increased anxiety was reported in the form of hospitalizations, 21% for the false-positive group versus 10% in the true-negative group. The authors suggested that mothers of the falsely-positive infants held altered perceptions of their child’s health because of their increased stress (7).

Screening Assays

Advances in technology have improved the detection methods used for newborn screening over the last 50 years. Half a century ago, each disorder was screened by its own test.
This was achieved by punching disks from the dried blood filter paper specimen and using one disk per test (7). Tandem mass spectrometry (MS/MS) is a new technology that is revolutionizing the newborn screening process by expanding the list of detectable conditions that had historically not been identified (1). This is achieved using a single disk from the blood spot on the filter paper (7). Although MS/MS is not a new technology, its use for NBS is a relatively recent development (1). It was first used in IEM detection (specifically MSUD) in the early to mid 1990s (9, 11) in Massachusetts, Pittsburg, and a few other countries, namely Australia, Germany, and Japan (1). There are several additional benefits of the new MS/MS technology.

Tandem mass spectrometry can screen for about 20-25 different disorders in a single test, including organic acidemias, disorders of fat metabolism, amino acidopathies, and hemoglobinopathies (1, 12, 13). This represents a large expansion over the original NBS program which used bacterial assays to look for 1 disorder per specimen (12). The benefit of detecting multiple conditions at once translates to cost savings (13). Before this technology, NBS expansion meant adding an extra test, which in turn led to increased costs (1).

Another advantage of MS/MS is its sharp reduction in the number of false-positive results. The NeoGen Screening program in Pittsburg was among the first to develop the MS/MS technology for use in NBS programs. They boasted a false-positive rate of 0.26% in 1998. At the same time, the New England NBS program reported a false-positive rate of 1.5% with its use of bacterial assays, which screened for significantly fewer diseases (12). This reduction is possible because of the ability of MS/MS to analyze metabolite patterns and ratios. This is in contrast to previous assays which simply look at the concentration of a single metabolite (1).

The MS/MS technology has made possible the detection of several conditions missing in a standard screen. For example, it is ideal for identifying the presence of homocysteinuria
because it is more sensitive to methionine than the traditional bacterial assay (1). Perhaps the most striking benefit of the use of MS/MS for NBS programs is the capability to detect fatty acid oxidation (FAO) disorders, which previous screening methods lacked the capacity to accomplish (14). However, two disorders typically screened for, galactosemia and biotinidase deficiency, are not detected using MS/MS but rather by other methods using the same sample. For example, multiple carboxylase deficiency (a result of a biotinidase deficiency), is detected by a colorimetric enzyme assay as opposed to MS/MS (6).

Overall, it is important that newly-developed, highly-sensitive screening methods avoid an increased number of false-positives. It is also becoming more and more important to utilize very sensitive tests to screen for these genetic conditions very early, namely because babies are now being discharged as soon as 1 or 2 days after birth (1). Tandem mass spectrometry is sensitive enough to provide the capability to make diagnoses from samples collected within the first 24 hours of life (9). To date, MS/MS has been the most successful means of meeting the goal of identifying all affected newborns while minimizing false-positives (1).

Several studies have been conducted to ascertain the validity of MS/MS in its application to newborn screening. One investigation conducted by Chace et al. tested the efficacy of MS/MS at diagnosing PKU while decreasing the number of false-positives in newborns less than 24 hours old as compared to fluorometry analysis (13). A total of 208 specimens collected in the early 1990s and analyzed by fluorometry were retrieved from storage (-20°C) and re-analyzed using MS/MS.

Tandem mass spectrometry was indeed effective in diagnosing PKU and its variant forms with 100% accuracy. Most importantly, it drastically decreased the amount of false-positives and demonstrated its ability to reduce the unnecessary work-up required of a false-positive result.
The original analysis of the 208 samples by fluorometry resulted in 91 false-positives. Tandem mass spectrometry correctly identified 88 of the 91 false-positive results as negative. Analysis of the phe to tyrosine (tyr) ratio further reduced the incidence of false-positives to 1. According to these results, MS/MS reduces the rate of false-positives to 1/100th of the rate of fluorometry (13).

Schulze et al. have further established the efficacy of MS/MS for expanded newborn screening in their analysis of 250,000 infants in Germany (14). Samples from both term and preterm neonates were collected between 3 and 7 days after birth. If an analyte was determined to be abnormal (13.8% of all samples), a second analysis was conducted of the same blood spot. If the second analysis was again abnormal (42% of the repeated tests), either another blood spot was obtained for testing or the infant was referred to a treatment center. This decision was made by an experienced metabolic specialist.

In this analysis, only 825 (0.33%) were found to be false-positive. This study boasts a false-positive rate for PKU of 0.05% which is remarkable when compared to the 0.23% false-positive rate using enzymatic phenylalanine determination. The overall false-positive rate of 0.33% for over 20 disorders in this study is comparable to that obtained from single-disorder screening tests for PKU alone. Tandem mass spectrometry was found to be 100% sensitive in detecting classic forms of metabolic disorders and 92.6% sensitive for variant forms. The authors concluded that MS/MS is an effective means of detecting the many known IEMs, which aligns with much of the available literature (14).

Because of the development of gene sequencing, Guthrie specimens can now be analyzed for DNA mutations. However, because so many genetic disorders are caused by DNA anomalies, diagnosis by DNA sequencing quickly would become cumbersome and expensive. Further, several conditions may be caused by multiple mutations. DNA analysis does, however,
offer a potential role in newborn screening by identifying genetic conditions undetectable by other methodology. Only a few such anomalies and conditions are currently known, such as the gene mutation responsible for childhood acute lymphoblastic leukemia. DNA analysis for mutations currently is used as a “second-tier” test in newborn screening. It is helpful in separating false-positive from true-positive screening results. Such analysis is currently used in some states for a few disorders including cystic fibrosis (CF), medium-chain acyl-CoA dehydrogenase deficiency (MCADD), and long-chain acyl-CoA dehydrogenase deficiency (LCHADD). Levy et al. explored in 2001 that second-tier testing might be used on a larger scale with DNA chip technology to detect hundreds of mutations in one analysis. Such an analysis would be conducted after an initial abnormal screening result, thus significantly decreasing the volume of false-positives and all the subsequent steps and anxieties (1).

In the early days of newborn screening, states were given the responsibility of developing their own standards and protocols (1, 4, 6). While this initially worked well, the disjointed set-up is now the cause of gaps and disparities in the disorders screened, the testing process itself, and the quality of follow-up (1). Each state’s screening panel is determined by the needs of that state’s population. A national standard for genetic screening for all newborns regardless of health status (preterm vs. term, healthy vs. sick) simply does not exist at this time (4). Furthermore, the number of conditions screened varies from 4 to 40 depending on the state policy (15). Every U.S. state screens for PKU and congenital hypothyroidism. As of 2005, every state except Washington also screened for galactosemia. Other screening tests, such as those for MSUD, homocystinuria, and biotinidase deficiency, vary in availability by state (4).

The existing lack of uniformity in newborn screening across the United States has resulted in significant disparities in the screening of newborns. There are several factors that
have inhibited the development of a standard national NBS panel, namely the resources available in each state including technological, monetary, and personnel variability. Legislation has also played a role in many states’ screening panel. Further, there seems to be significant limitations with regards to the infrastructure and connectedness among health care professionals when it comes to managing genetic disorders. This lack of cohesion is particularly pronounced in rural areas. Providing the best possible care to an affected newborn should be the number one priority in the development of NBS policies in the future (3).

Recently, an initiative by the American College of Medical Genetics to standardize the newborn screening panel has resulted in the suggestion that a core panel of conditions be tested. The Newborn Screening Expert Group has conducted a large-scale review of the current screening system and has condensed their findings into an executive summary. According to this assessment, 29 genetic conditions should be included in the NBS panel in every state. These 29 were recommended because screening assays have already been developed, effective treatments are available, and because they are sufficiently understood. Nearly all (23 of 29) of the conditions on this proposed panel can be detected with new and sophisticated “multiplex technologies” like MS/MS. Included are 9 different organic acidurias along with 6 amino acidopathies, 5 FAO disorders, 3 hemoglobinopathies, and 6 other conditions (3). Two of these final 6, citrullinemia and argininocuccinic acidemia, are urea cycle disorders. Disorders affecting the other 4 enzymes in the nitrogen pathway of the urea cycle were not advised for inclusion in the core panel because they either were a component of the differential diagnosis of one of the conditions in the core panel (and thus considered as a secondary target for screening), or there is no effective population-based screening test available (6).
A move to the recommended core screening panel will represent an expansion for many NBS programs. Pia Hardelid and Carol Dezateux support this expansion, citing the success of PKU screening as their basis. They emphasize that a well-structured system is essential to the prevention of neurological morbidity (16). George Cunningham, with the Disease Branch of the California Department of Health Services, has responded to critics of NBS expansion who assert that cost is the limiting factor. He has emphasized that preserving the quality of life of every individual with an IEM is worth all necessary expenditures (17). Such criticism, in fact, may not be well-founded. Cost-benefit analyses conducted by the NBS Expert Group show that most newborn screening programs are indeed successful in reducing expenses while improving outcomes. This cost-benefit analysis also validates the continued use of multiplex screening technologies like MS/MS because of their ability to screen for multiple conditions at once while reducing false-positives (3).

The specificity and accuracy of MS/MS in newborn screening is beneficial in that fewer diagnostic work-ups must occur in response to false-positive screening results, thereby reducing both costs and anxiety. Diagnosis of an IEM requires multiple medical procedures that may include repeat screening, urine tests, additional specialized blood analyses, clinical evaluation, and genetic testing. For a few conditions, diagnostic testing can only be performed by one or two labs in the world. While actual monetary figures that assess the total price of false-positives have not been reported, one can speculate the cost-savings that can be achieved through avoidance of these added expenses. This can be illustrated by looking at the cost of a newborn screening test alone. Some states charge as much as $139 per blood spot analysis (18). To further illustrate, it is helpful to assess the cost of specific laboratory tests that are common during a diagnostic workup. The Mayo Clinic’s Medical Laboratory is capable of many
specialized diagnostic blood and urine tests and handles the specimens sent to them from many metabolic programs. They report that the lab cost for a plasma acylcarnitine profile to assess for the presence of a FAO disorder is $233.40. Tests can be even more costly, demonstrated by the price of a quantitative amino acid urine analysis used to diagnose an amino acidopathy: $718.20 (19). These fees do not include surcharges applied by the referring hospital. Repeating newborn screens and/or amassing additional diagnostic tests can quickly add to the overall medical cost burden.

Newborn Screening in the NICU

The information presented thus far has discussed generalized newborn screening. Special infant populations, specifically critically ill and preterm infants in the neonatal intensive care unit (NICU) setting, require a separate analysis. NICU patients actually more often experience false-positive NBS results than patients in newborn nurseries. In fact, up to 40% of all abnormal NBS tests come from NICU patients (2). Presently, specific rates of false-positives in Virginia, as well as data comparing screening results in the NICU versus the wellborn nursery, have not been compiled (20). However, certain issues that contribute to the problem of false-positives in this population have been identified.

While there are numerous factors which influence the newborn screening results, these issues are exacerbated in the NICU population. First and foremost is the question of the timing of blood specimen collection. Different genetic disorders have different “screening windows” when there is the best chance for a diagnosis. A screening window is defined as “the period between when the abnormal analyte is detectable by newborn screening and the development of symptoms” (2). Because more and more genetic disorders are being discovered, it is becoming
quite difficult to choose a screening window that will catch most, or all, conditions with the NBS. Research supports that FAO disorders are best detected approximately 24-48 hours after birth but before parenteral nutrition (PN) is given because most babies are in a state of catabolism during this time. Specimens collected at 48-72 hours after birth are most reliable for severe congenital hypothyroidism (CH), congenital adrenal hyperplasia (CAH), pancreatic-insufficient CF, and most amino acidopathies. It is best to screen for hemoglobin disorders, galactose-1-phosphate uridyl transferase (GALT), and biotinidase disorders before any treatment is given, or using cord blood, as these disorders do not require accumulation of a substrate for detection. Blood analysis before any treatment is also the best time to determine baseline amino acid and acylcarnitine metabolism (2).

An early discharge from the hospital means that newborn screening specimens may be collected before the infant is even 24 hours old. In this case, the dependability of the screening results comes into question. Many genetic disorders cannot be detected very early after birth because the problematic metabolites have not had sufficient time to metabolize (1). In fact, Guthrie specimens taken before 24 hours of life are not diagnostic of amino acid disorders, especially if the results are normal (2). To illustrate, in utero, an infant with PKU maintains normal plasma phe concentrations because the substrate is passed across the placenta and metabolized by the mother. This results in nearly normal phe levels at birth and a subsequent accumulation over time (8). Because specimens are being collected sooner after birth, it has been suggested that the best way to avoid missing any affected infants screened is to lower cutoff levels for flagging abnormal specimens. This would ensure that any elevations of the metabolite in question (i.e. phenylalanine) can be detected (1). Such a move may or may not affect false-positive rates. Furthermore, screens taken sooner than 12-24 hours of life may result in either
false-positive or false-negative for thyrotropin (thyroid stimulating hormone; TSH), 17-
hydroxyprogesterone (17-OHP), and immunoreactive trypsinogen (IRT) (2).

Historically, the timing of discharge has affected the timing of NBS sample collection. The precedent began when PKU screening was first implemented in the 1960s as samples were taken at discharge, usually about 72 hours or later after birth (13). Currently, infants are being sent home much sooner after birth and thus decreasing the amount of time for problematic metabolites to accumulate. To remedy this, Goodin has suggested that blood samples for the analysis ideally be collected approximately 24 hours after birth and again just before the infant goes home (6). This recommended protocol, however, is not specific for the NICU population in which multiple factors can affect the NBS results.

Critically ill newborns, particularly those born prematurely, are at risk for missed or incomplete NBS tests for a variety of reasons. These include special treatments and other unique aspects of their care, their “pre- and post-natal environments,” and simply their altered metabolisms resulting from prematurity (2). Many standard medical care regimens in the NICU are also known to affect newborn screening results in one way or another. Such interventions include heparinized solutions, nothing by mouth (NPO) status, blood transfusions, and medications such as aminoglycoside and antibiotics (4, 15, 18). More specifically, blood products from healthy individuals can provide normal enzyme activity within red blood cells (RBCs) and therefore result in false-negative newborn screening results for about 120 days (the life span of a typical RBC) (2). Inadequate protein intake and/or NPO status is known to skew the results of the screening test for galactosemia, branched chain keto-aciduria, homocysteinuria, tyrosinemia, and MSUD (4). Additionally, adequate provision of nutrition, which encourages anabolism, may actually mask a FAO disorder as these often manifest in times of fasting or other
catabolic states (2). Such a broad range of confounding factors certainly makes standardized screening protocols difficult to develop. In addition to the above, the nutrition delivered to preterm and critically ill infants may also play a role.

**Nutritional Support**

The high-risk newborn has an immediate need for nutrient delivery after birth because of their limited glycogen and fat stores as well as their high nutrient requirements for growth (21, 22). Ideally, enteral nutrition (EN) support is initiated by day-of-life 7 and infused at a minimum rate of 10-20 ml/kg/day for 3-5 days either continuously or intermittently. Depending on weight and acuity, feedings of either human milk or specialized preterm formula can be advanced by 20-35 ml/kg/day until goal intake is achieved (23). In many cases, EN is not achievable and therefore parenteral nutrition must be utilized. It is this type of nutrition support that is suspected of interfering with the newborn screen.

Maximum parenteral protein administration of 3 g/kg/day as early as possible can help with protein deposition and therefore improve early growth. Very premature babies may need more to achieve the same result, up to 4 g/kg/day (21). However, different PN solutions can elicit a variety of conflicting results in the NBS. It is known that PN affects amino acid (AA) concentrations on a newborn screen, typically by causing higher-than-normal circulating levels. Conversely, some amino acids may be decreased after PN has been started. It is unknown how long that impact lasts. A screening lab can often discern a PN effect from a true metabolic disorder depending on the ratios of the elevations. For example, the ratio of phe:tyr can distinguish PKU from PN. Elevations in both AAs likely represents PN administration while an increased phe with a low tyr level indicates PKU or one of its variants. Additionally, intravenous
lipids in PN can also influence NBS results, although the exact mechanisms have not been well-studied. This is significant because lipid in PN is often prescribed in the first day of life in NICU patients to prevent essential fatty acid (EFA) deficiency and to effectively increase calorie intake (2, 18).

The impact of parenteral nutrition on newborn screening has not yet been well studied although some associations are known. What is known is that PN can cause a false-positive in screening for amino acidopathies, organic acid disorders, and urea cycle disorders. Carnitine supplementation in preterm infants can actually cause an elevation in acylcarnitines and a decrease in long-chain acylcarnitines as compared to healthy newborns. This results in either a false-positive or –negative in FAO disorder screens. Additionally, L-carnitine given in PN, which is often administered to facilitate transport of fatty acids into the mitochondria for energy production, can essentially hide a carnitine transport disorder (2, 22). The effect can last for weeks after supplementation ceases (2).

Both prematurity and critical illness are known to cause adverse effects on some disorders such as CF, hemoglobinopathies, and some enzymopathies (i.e. galactosemia). The care provided in these situations also plays a role. However, effects on most metabolic disorders have yet to be described (2). Because of their relatively large mass of metabolically-active organs, preterm infants are metabolically very different than term infants. This impacts their nutritional needs for growth and is related to their low energy stores, high rate of protein flux, and overall increased metabolic rate (24). As mentioned above, one factor that puts preterm infants at such high risk is their limited energy stores. Only 2% of their body weight is fat and glycogen makes up less than 0.5%. In comparison, a healthy term infant has 15% and 1.2% respectively (25).
Research on the metabolic differences between term and preterm infants remains limited. Most of what is known has been discovered as a result of monitoring tolerance to different formulations of nutrition support. In utero, amino acids are the main source of energy and growth for the fetus. The administration of IV amino acids can help prevent catabolism when given very soon after birth in preterm infants (21). Murdock and colleagues demonstrated in 1995 that preterm infants can in fact tolerate macronutrient infusions immediately after birth. They concluded that the interruption of nutrient administration between gestational and postnatal periods should be minimized and can be done so safely (26). Thus the recommendation is to begin amino acid infusion within the first 24 hours of life in infants who require parenteral nutrition support. Other studies have shown that this early infusion of nutrition will improve nitrogen balance and glucose, thereby decreasing the time needed on parenteral nutrition, and in turn reducing costs (21). However, it is pivotal to keep in mind the effects of PN support on the newborn screen when reviewing these studies.

A conservative approach to early infusion of amino acids has historically been taken because of the concern that the preterm infant has difficulty metabolizing them. Interestingly, studies done to assess this by monitoring for hyperammonemia, azotemia, and metabolic acidosis have not found such complications (24). Many practitioners assert that the conservative approach had grown out of the complications that surrounded early formulations of IV amino acids. However, it is well documented that these metabolic effects occur less frequently with newer formulations (26-28).

Aside from amino acid intake, energy intake and various disease states can also affect protein metabolism (29). Many studies have been done to determine the exact relationship. Thureen et al. found that preterm infants receiving high amounts of IV amino acids (2.65 g/kg/d;
n=15) had elevated AA levels compared to preterm infants receiving a lower AA concentration (0.85 g/kg/d; n=13) approximately 24 hours after birth (24). These blood elevations were actually found to be lower than or similar to those of the unborn fetus. They demonstrated that the infants had improved protein accretion with the higher AA infusion as evidenced by both nitrogen balance studies and leucine isotope methodology which suggested that these elevations in circulating AA levels are actually helpful rather than harmful. Their results imply that parenteral AA infusion actually stimulates protein synthesis as opposed to merely suppressing protein breakdown.

Extremely low birth weight (ELBW) infants lose approximately 1% of their protein stores every day that they do not receive a nutritional source of protein. This occurs even with IV glucose administration. However, even ELBW infants seem to be able to utilize protein intake as effectively as healthy term infants (29).

In a study assessing protein kinetics in preterm infants, Goudoever found that infants who received only glucose (4.5±0.9 mg/kg/min; n=9) had negative nitrogen retention (-110±44 mg/kg/d) (27). This was statistically significant in contrast to the nitrogen retention of the intervention group (10±127 mg/kg/d) who received both glucose and amino acids (4.2±1.1 mg/kg/min glucose, 0.8±0.04 mg/kg/min protein; n=9) as measured by urinary nitrogen excretion (p=0.001). The researchers found that plasma phe levels were elevated in infants who received AAs, yet this elevation was considerably lower than the concentration typically seen in classic PKU (<10%). Plasma cysteine concentrations were low even in infants who received AAs. Overall, total AA concentrations in the preterm infants studied resembled those of unborn fetuses during the 2nd and 3rd trimester. Other research agrees that an elevated phe level can be associated with prematurity rather than a genetic abnormality (1).
The results of a study conducted by te Braake et al. found that very early infusion of AAs (2.4 mg/kg/d; 2 hours after birth) actually normalized the plasma concentrations of most AAs in preterm infants weighing less than or equal to 1500g (n=66) when compared to similar infants who received amino acids starting on day-of-life (DOL) 2 (1.2 mg/kg/d on DOL 2, 2.4 mg/kg/d on DOL 3; n=69) (28). The authors asserted that elevated BUN levels in preterm infants should be considered as a marker of AA oxidation rather than protein intolerance.

It is hypothesized that insulin, in addition to amino acids, also helps to regulate protein accretion in preterm infants. Exactly how this happens is unknown for this population. Additional results from Thureen’s evaluation in very preterm infants, in conjunction with those of published animal studies, suggested that higher insulin levels (related to higher AA concentrations) aid in protein deposition (24).

Another study conducted by Clark et al. contradicts the evidence that very preterm infants adequately tolerate early parenteral nutrition infusions (30). They hypothesized that providing too much protein can saturate the metabolic pathways in preterm infants because of their limited metabolic capacity and result in excessive AA pools that take time to clear. They measured blood AA levels in preterm infants (23 weeks 0 days to 29 weeks 6 days gestation) receiving 2 different IV protein administration regimens (goal 2.5 g/kg/d; n=58 or 3.5 g/kg/d; n=64). One week into the study period, some AAs (alanine, glutamine, leucine, isoleucine, methionine, phe, serine, tyr, valine, and ornithine) were found to be present in higher concentrations in the higher-protein group when compared to the lower-protein group. Interestingly, some of these were considered low when compared to the healthy infants in the reference sample (alanine, glutamine, tyr, and phe). By contrast, some were higher than those in healthy infants including arginine, leucine, isoleucine, methionine, and ornithine. Some AA levels were above the 90th
percentile on average in both treatment groups while some averaged below the 10th percentile when compared to healthy newborns. Several acylcarnitines were found to be low in the treatment groups when compared to healthy term infants. This difference was found on all 3 days of blood spot testing (enrollment day and days 7 and 28 of life). The data suggested that the leucine metabolic pathway quickly becomes saturated in premature babies and that higher doses of this AA would lead to accumulation in the blood. It is difficult to interpret the results of this study’s AA supplementation in preterm infants on a large scale because no standard reference exists. Each state develops its own references for normal versus abnormal AA blood concentrations. The authors concluded that very premature newborns may need special consideration and individualization as to the type and amount of IV amino acids they receive. Furthermore, there is no data within this study that suggested a correlation between blood AA levels and newborn screening results.

One must also consider that lipid metabolism in this special population is altered. Preterm infants provided with lipids avoid the development of an EFA deficiency, which quickly occurs without dietary fat. Preterm infants have decreased carnitine production and often require supplementation to adequately metabolize long-chain fatty acids. Premature and malnourished babies have less endothelium tissue. This is significant because endothelium releases lipoprotein lipase. The result is a reduced ability to process fat in these infants. Therefore, IV lipids are better tolerated when infused over 24 hours in these patients as opposed to shorter time periods. Tolerance can be monitored by assessing serum triglyceride (TG) levels (21).

As previously discussed, little is known about what factors interfere with newborn screening results in the NICU population. Cause-and-effect research is implicitly needed to help revise NBS protocols (2). Currently, there is no recommended screening guideline that is
specific to this special population. In general, the optimal time to collect Guthrie samples is between 48-72 hours after birth. This is the time when disorders can be detected from metabolite accumulation (9). Ideally, very low birth weight (VLBW) premature babies should be screened for urea cycle disorders, organic acidurias, and amino acidopathies about 24 hours of age and before PN is given. However, a single NBS specimen in any NICU infant is not sufficient to properly rule in or out an IEM (2). Balk advocates for a change in the NBS policy within all NICUs as an effort to further reduce missed metabolic diagnoses. This change entails 2 separate screens: one at birth before any intervention, and a second on day 7 of life. If possible, a final screen should be done around DOL 28 (15). Standardization and expansion of NBS guidelines remains warranted.

The Problems Inherent with False-Positives

Cutoff values for each metabolite analyzed must be set with 2 goals: to avoid false-negatives (by not setting the value too high) and minimize false-positives (by not setting the value too low) (1, 18). Simply, programs want to avoid missing any diagnoses but also avoid the costs and anxieties associated with false-positives.

A comprehensive look at NBS data from 1993 and 1994 revealed that over 90,000 abnormal initial screens in each year were found to be falsely-positive (number of total screens assessed was not given). Comparing these numbers to the number of confirmed cases yielded a more than 50:1 ratio when looking at 5 specific disorders. False-positive screening results present a number of problems. Higher incidences of false-positives translate to more repeat tests and therefore higher costs incurred (31). A significant proportion of the cost of newborn
screening programs goes toward the work-up of false-positive results. Additionally, it also creates considerable stress among parents and healthcare workers alike (12).

Every abnormal screening result initiates a cascade of events. The first step is to repeat the test of the original specimen and/or utilize second-tier testing. If the result is again abnormal, the child’s physician or the health care facility is notified and responsible for obtaining another Guthrie specimen. Depending on the metabolite and the level, this request may or may not be emergent in nature. Regardless of the urgency, much anxiety and worry often is apparent at this stage, particularly within the parents of the affected child. The repeat specimen must then be re-analyzed. Often, this second screen is normal. If not, diagnostic work-up must begin (1). It may be difficult to obtain a second sample for a repeat screen. Historically, many parents fail to provide a second blood specimen from their child for repeat testing for unclear reasons (31). Some states have attempted to rectify this problem by collecting a second blood sample at the first well-baby check up. Infants who remain hospitalized at the time of the repeat screen are at risk of a second abnormal result simply because of procedural errors that occur during the collection. The repeat blood sample may be mistakenly drawn from the same IV line through which the parenteral nutrition is infusing. Ideally, IV amino acids should be stopped at least 1 hour prior to a second blood specimen collection (18).

A false-positive result can clearly elicit undue psychological stress for the parents and families of children who require repeat testing (31). In 1984, Sorenson et al. published results from their assessment of parental anxiety related to repeat newborn screening. Although they were not compared to parents of infants with normal NBS results, parents whose children required re-screening understandably demonstrated concern over their child’s health. However,
parents who were well-informed did not seem to be as “psychologically upset” over the repeat screen. Many parents enrolled in this study reported some anxiety over the repeat screen (10).

It is fairly common for preterm infants to have a temporary abnormality that surfaces on a newborn screen (7). Again, NICU patients more often experience false-positive NBS results than patients in newborn nurseries. Between 2 and 10% of NBS tests are abnormal. Of these, up to 40% come from NICU patients. This results in a need for more thorough work-up that often leads to the discovery of a false-positive screen (2). A mere 5% of the more than 160,000 infants screened in a study by Zytkovicz et al. were from the NICU/LBW population (32). A striking 50% of the abnormal screening results were from this small group. These remarkable results provide strong evidence of the need for special screening procedures in this unique population. Further, the authors reported that 21% of all infants diagnosed with a metabolic disorder were from the NICU/VLBW population.

Summary and Purpose

Overall, the newborn screening program has been quite successful in reducing the morbidity and mortality associated with inborn errors of metabolism. However the significant gaps in research conducted to date have left health care providers with many questions, specifically when it comes to detecting IEMs in the NICU population. To date, no research has been done that looks explicitly at trends among NICU patients between NBS results and nutritional support. Do preterm and critically ill infants on PN actually pose a higher risk of an abnormal screening result or does that risk come from non-nutritional origins? Is there a way to predict a false-positive NBS result based on the nutritional intake of the infant? It is the primary purpose of this proposed study to begin to answer such questions.
References

Chapter 3: Nutrition Support and Newborn Screening in the NICU Population: Is There A Link?

Abstract

Background: Recent research is revealing the high rate of false-positive screening results for IEMs in the NICU population. No study published to date has specifically studied the possible relationship between nutrition and newborn screening in this population.

Objective: It is suspected that NICU infants who receive PN are more likely to have abnormal newborn screening results than infants who receive EN. An understanding of the role of nutrition will assist in developing protocols for screening in the NICU and decrease false-positives.

Design: Infants admitted to the NICU between January 1-June 30, 2009 were included in this retrospective chart review study (n=339). The type of nutrition and timing of its initiation was recorded and compared to newborn screening results to identify correlations with false-positives. Statistical analysis included means, percentages, Fisher’s exact test, Chi-square test, and the Cochran-Mantel-Haenszel test.

Results: Nutrition type was significantly associated with newborn screening (p<0.001); those who received parenteral nutrition were more likely to have a false-positive. For infants who also received PN, EN of breast milk exclusively increased risk of an abnormal screen more than formula exclusively or breast milk plus formula. The timing of parenteral nutrition had no effect on screening. Premature infants who received PN exclusively had a higher percentage of false-positives than those who received EN.
Conclusions: Although the hypothesis could not be statistically supported, PN appears to contribute to false-positive newborn screens. More research is needed to ascertain the role of EN and GA in newborn screening and to develop standardized protocols.

Introduction

There is no greater example of the balance between the healthy and adverse effects of food as that surrounding Inborn Errors of Metabolism (IEMs), in which proper, specialized nutrition is essential to normal growth and development. Unfortunately, there are numerous challenges of diagnosing these disorders in the premature and critically ill newborn population due to factors such as specialized medical care, nutritional support, and the current lack of understanding into their unique metabolic processes. Furthermore, the actual screening process used to detect these conditions has inherent limitations. The problem of false-positive test results on the newborn screen has become apparent in recent years and little research has been done to discern the effects of various factors on the results. There is a clear need to establish the relationship between nutritional support in the preterm and critically ill infant population and the newborn screening blood analysis for IEMs.

Inborn Errors of Metabolism are genetic defects that alter the processing of certain nutrients within the affected individual, specifically macronutrients. Each disorder affects a particular biochemical pathway or pathways within the body with a wide variety of outcomes ranging from no clinical effect, to permanent neurological damage, to death. Many of these conditions can be successfully treated without adverse outcomes if discovered early and treatment is initiated immediately. Table 3 outlines the different types of IEMs and some of the specific disorders that are known. An IEM is diagnosed once in every 4,000 live births in the
United States (1). The frequencies of specific conditions can be tracked through the Genetics Home Reference webpage maintained by the U.S. National Library of Medicine (2).

Table 3: Types of Inborn Errors of Metabolism

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<thead>
<tr>
<th>Organic Acidemias</th>
<th>Amino Acidemias</th>
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<tr>
<td>Isovaleric Acidemia (IVA)</td>
<td>Phenylketonuria (PKU)</td>
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<tr>
<td>Glutaric Aciduria Type 1 (GA-1)</td>
<td>Maple Syrup Urine Disease (MSUD)</td>
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<tr>
<td>Multiple Carboxylase Deficiency</td>
<td>Homocystinuria (HCY)</td>
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<tr>
<td>Propionic Acidemia (PA)</td>
<td>Tyrosinemia Type 1 (TYR-1)</td>
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<tr>
<td>MethylMalonic Acidemia</td>
<td>Arginosuccinic Acidemia (ASA)</td>
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<tr>
<th>Hemoglobinopathies</th>
<th>Other Disorders</th>
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<tbody>
<tr>
<td>Sickle Cell Anemia (Hb SS)</td>
<td>Congenital Hypothyroidism (CH)</td>
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<tr>
<td>Hemoglobin S-beta-Thalassemia (Hb-SβTh)</td>
<td>Biotinidase Deficiency (BIOT)</td>
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<tr>
<td>Hemoglobin SC Disease (Hb S/C)</td>
<td>Galactosemia</td>
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<td></td>
<td>Cystic Fibrosis (CF)</td>
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<tr>
<th>Fatty Acid Oxidation Disorders</th>
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<tr>
<td>Medium-Chain Acyl-CoA Dehydrogenase Deficiency (MCADD)</td>
</tr>
<tr>
<td>Long-Chain L-3-Hydroxyl Acyl-CoA Dehydrogenase Deficiency (LCHADD)</td>
</tr>
<tr>
<td>Very Long-Chain Acyl-CoA Dehydrogenase Deficiency (VLCADD)</td>
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<tr>
<td>Carnitine Uptake Defect (CUD)</td>
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Table derived from Goodin (3) and Balk (4)

Population-based newborn screening (NBS) has been established as a preventive public health measure available to all neonates. It began in the 1960s with a single test to screen for phenylketonuria (PKU) (5). Over the last 50 years, NBS has expanded to include more than 20 conditions (6). This is much in part due to advancements in screening technologies such as tandem mass spectrometry, which has been well-validated for its use in newborn screening (7-11). Identification of an IEM is achieved by detecting high circulating levels of certain metabolites, specifically amino acids.

Each state is responsible for developing and maintaining its own NBS system, which includes screening, diagnosis, education, crisis management, and follow-up throughout an individual’s lifetime (7, 12). The lack of uniformity between state screening panels has resulted in significant disparities in the screening of newborns and has thus prompted the American College of Medical Genetics to review the process and recommend a core screening panel of 29
genetic conditions (12). Their recommendation is a step toward a national standard in newborn screening.

False-positives occur when a newborn screen shows the presence of a metabolic abnormality that suggests a genetic disorder when in fact that condition is not present. Frequently, they are transient findings caused by biochemical or hormonal fluxes as opposed to lab error (6, 13). The costs associated with a false-positive can quickly accumulate and include repeated screens and diagnostic testing (14). Furthermore, much anxiety can accompany a false-positive, particularly for the parents of the infant (6, 8, 13).

Premature and critically ill infants in the neonatal intensive care unit (NICU) represent a unique challenge to newborn screening. These patients more often experience false-positive NBS results than patients in newborn nurseries. Between 2 and 10% of all NBS tests are abnormal; of these, up to 40% come from NICU patients (15). A study conducted by Zytkovicz et al. found similar results. A mere 5% of the more than 160,000 infants screened in their study were from the NICU population. A striking 50% of the abnormal results were from this small group (11). Several factors may contribute to the substantial rate of false-positives in this population, most notably nutrition support. The high-risk newborn has an immediate need for nutrient delivery after birth because of their limited glycogen and fat stores as well as their high nutrient requirements for growth (16, 17). These infants often receive enteral nutrition (EN), parenteral nutrition (PN), or both. The administration of PN often causes higher-than-normal circulating levels of amino acids (15). To date, the factors that affect newborn screening results, including feeding modalities, have not been well studied.

Clearly, research evaluating the possible relationship between nutrition support and its influence on the newborn screen is warranted. The primary goal of the present study was to
identify correlations between the nutrition provided to the NICU patient and abnormal results on the newborn screen, particularly, which factors may contribute to false-positive results. It is hypothesized that NICU infants who receive PN are more likely to have abnormal results on the newborn screen for amino acidemias, organic acidemias, fatty acid oxidation disorders, and galactosemia than infants who receive enteral nutrition with either formula or breast milk.

A deeper understanding into the causative factors for false-positives will aid health care providers in predicting and possibly avoiding the costly and stressful work-up required to rule in or out a metabolic disorder. It may also assist with the development of standardized protocols for nutrient delivery and blood sample collection and therefore consistent and optimal screening procedures across the country, specifically for the NICU population. Additionally, it may become possible to predict false-positive NBS results and thus avoid the expensive and stressful process of diagnostic workup. The data collected in this study will provide the basis for a full-scale assessment of the influence of nutrition support in newborn screening.

Materials and Methods

This retrospective study was conducted at Carilion Roanoke Memorial Hospital (CRMH), an 825 bed tertiary care center located in Southwest Virginia. Approval from Carilion’s Institutional Review Board was obtained prior to the study commencement. Carilion RMH houses a 60 bed NICU and cares for over 500 preterm and critically ill newborns each year (18). Newborn screening blood specimens are collected by day-of-life (DOL) 7 or before the first blood transfusion and sent to the Division of Consolidated Laboratory Services in Richmond, Virginia for analysis. Carilion utilizes an electronic medical record for patient care
documentation called Epic. This program, along with the information found in the paper medical charts, was used for data collection.

Infants admitted to the NICU between January 1, 2009 and June 30, 2009 were included in the data collection (n=339). Subjects who did not survive to discharge were excluded from the analysis. Information collected was entered into a secure data collection program called SoftMed that is used by the Carilion system for research and other quality improvement purposes. Specific items evaluated included birth weight, gestational age (GA), type of nutrition provided, time of nutrition initiation (hours after birth), NBS results [normal or abnormal for the amino acid profile, fatty acid oxidation (FAO) profile, organic acidemia profile, and galactosemia screens], and timing of NBS specimen collection. Results for the biotinidase, congenital adrenal hyperplasia, hemoglobinopathy, cystic fibrosis, and thyroid disorders were excluded from the analysis. The results of repeat screens and any diagnostic work-up were included in the outcomes measures as a way to discern a false-positive from a true-positive. See Appendix 2 for a list of the SoftMed data fields collected.

Intake and output patient flowsheets were reviewed in Epic to determine the timing of nutrition intake. All forms of nutrition provided prior to the blood sample collection were recorded separately. Epic was also used to extract birth time and weight. The paper charts contained the actual NBS result sheets from the Division of Consolidated Laboratory Services where blood sample analysis had been conducted. Information from the paper charts was also used to verify the nutritional plans for each individual infant included in the study. This served as a way to validate the accuracy of the flowsheets in Epic.

The timing of the first properly-collected NBS result was recorded as well as information about the infant’s nutritional intake prior to the sample blood draw. Collection was considered
appropriate if it was taken at least 24 hours after birth and after the beginning of nutritional intake. All possible forms of nutritional intake were considered, including by-mouth (PO), tube feeding, and intravenous (IV) feeding. Infants may have received any combination of the 3 forms of intake with a variety of types of formulations, including breast milk, infant formula, IV protein, and IV lipid. Typically for PN, an IV protein and glucose solution was administered very soon after birth, followed by the addition of lipids on the second day-of-life. Based on the review of the literature, glucose has not been implicated in abnormal blood metabolite levels. Further, the critical conditions of the study population often required very early IV glucose administration, making it difficult to determine the exact timing of its initiation based on the medical records. Therefore, IV glucose administration was excluded from the data analysis.

Many infants were screened multiple times for reasons such as improper blood sample collection (i.e. less than 24 hours of age) or follow-up on abnormal results for a condition not considered in the present study (i.e. hemoglobinopathies). If a false-positive screening result for the disorders examined in the present study was found on a second or subsequent NBS blood draw, the previous results were discarded and all nutritional intake information reflected what the infant received prior to the abnormal screen. This was done in an effort to capture all nutritional factors that may have contributed to the result.

Statistical analysis was completed using SAS version 9.1.3 (SAS Institute, Inc, Cary, NC, 2009). Descriptive statistics included means and standard deviations for continuous variables and counts and percentages for categorical variables. Contingency table analysis with chi-square was used to test associations between the categorical variables, which were all nominal. Fisher’s exact test was used for crosstabulations with zero cells or sparse data. In the case of stratified
analysis of categorical variables while controlling for a third variable, the Cochran-Mantel-Haenszel statistic was used. A p value of less than 0.05 was considered significant.

Results

Out of the 339 charts reviewed, the data from 66 infants were not included in the study. Reasons for omission were: no newborn screening record on chart (n=17), incomplete nutritional intake records from transferring hospital (n=13), NBS completed at transferring hospital and results not sent to CRMH (n=10), deceased (n=8), poor documentation (n=6), improper NBS collection without repeat screen (n=5), no nutritional intake before the only NBS collection (n=3), other pertinent information missing (n=2), proper NBS sample collected after hospital discharge (n=1), and infant was not a newborn (n=1). The final sample included in the data analysis consisted of 273 infants. Diagnoses varied widely and included gastroschisis, hypoglycemia, intra-uterine growth retardation, maternal drug use/infant withdrawal, respiratory distress, macrosomia, cleft palate, tracheoesophageal fistula, and congenital bowel obstruction, among others.

Sample Characteristics

The mean birth weight of the infants in the study was 2135.5g (range 557-6116g). A total of 114 infants were classified as low birth weight (LBW; 1500-2500g), 43 were very low birth weight (VLBW; 1000-1500g), and 28 were considered to be extremely low birth weight (ELBW; <1000g), leaving 88 infants who were born at or above normal weight. A total of 208 infants (76.2%) were born before 37 weeks gestation and were therefore considered preterm.
Nutrition Intake

Only 37 infants (13.6%) received PN exclusively prior to their newborn screen compared to 71 (26%) who received EN exclusively. A total of 165 subjects (60.4%) received a combination of PN with EN. The breakdown of the combinations of nutrition provided to the infants and how many received each combination is shown in Table 4. Of the infants who received PN, IV protein was initiated at an average age of 14.5 hours (range 0.6-76.1 hours). Intravenous lipid was typically added to the PN later at an average of 44.5 hours after birth (range 11.1-101.3 hours). The mean age at initiation of EN was 39.2 hours for the 236 infants who received it prior to the NBS blood sample collection (range 0.9-279 hours).

Table 4: Combinations of Nutrition Provided to Infants

<table>
<thead>
<tr>
<th>Nutrition</th>
<th>EN Only</th>
<th>PN Only</th>
<th>Both EN + PN</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EN Provided</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast Milk Only</td>
<td>5</td>
<td>--</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>Formula Only</td>
<td>40</td>
<td>--</td>
<td>66</td>
<td>106</td>
</tr>
<tr>
<td>Breast Milk + Formula</td>
<td>26</td>
<td>--</td>
<td>79</td>
<td>105</td>
</tr>
<tr>
<td><strong>PN Provided</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV Protein Only</td>
<td>--</td>
<td>9</td>
<td>13</td>
<td>22</td>
</tr>
<tr>
<td>IV Lipid Only</td>
<td>--</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IV Protein + IV Lipid</td>
<td>--</td>
<td>27</td>
<td>152</td>
<td>179</td>
</tr>
<tr>
<td><strong>Total per Nutrition Type</strong> (n=273)</td>
<td>71 (26.0%)</td>
<td>37 (13.6%)</td>
<td>165 (60.4%)</td>
<td></td>
</tr>
</tbody>
</table>

False-Positives

There were a total of 23 false-positive newborn screenings representing 8.42% of the total final sample. Twelve infants were flagged for amino acidopathies, 8 for FAO disorders, 2 for galactosemia, and 1 for organic acidemia. No IEM was diagnosed in the study group. The false-positive was not necessarily from the first properly-collected blood sample; 10 infants were flagged from a second or third blood sample analysis. Two infants were flagged on separate NBS blood draws for 2 types of IEMs (amino acidopathy and FAO disorder). Each of these
infants was only counted as 1 false-positive and the nutritional information reflected the intake prior to the first properly-collected abnormal screen. There was no association between the type of nutrition provided and the disorder flagged on the NBS. The specific breakdown is described in Table 5.

**Table 5: Disorders Flagged and Nutrition Intake Type**

<table>
<thead>
<tr>
<th>Nutrition Type</th>
<th>Amino Acidopathy</th>
<th>Fatty Acid Oxidation</th>
<th>Galactosemia</th>
<th>Organic Acidemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parenteral Nutrition</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Enteral Nutrition</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Both EN &amp; PN</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Totals</td>
<td>12</td>
<td>8</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

In order to answer the primary research question, a chi-square test was conducted to see if there was an association between type of nutrition (PN versus EN versus both) and the incidence of false-positives. The result was significant indicating that type of nutrition and incidence of false-positives are associated ($p < 0.001$). Table 6 contains the breakdown of the nutrition provided to the infants in this group. In the group of neonates who received EN only, 1.4% had a false-positive compared to 7.9% in the EN plus PN group. However, 24.3% of the group who received PN only had a false-positive on the newborn screen.

**Table 6: Combinations of Nutrition Provided to Infants with False-Positive NBS**

<table>
<thead>
<tr>
<th>Nutrition</th>
<th>EN Only (n=71)</th>
<th>PN Only (n=37)</th>
<th>Both EN + PN (n=165)</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EN Provided</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast Milk Only</td>
<td>0</td>
<td>--</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Formula Only</td>
<td>0</td>
<td>--</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Breast Milk + Formula</td>
<td>1</td>
<td>--</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td><strong>PN Provided</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV Protein Only</td>
<td>--</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>IV Lipid Only</td>
<td>--</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IV Protein + IV Lipid</td>
<td>--</td>
<td>7</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td><strong>Total per Nutrition Type</strong> (n=23)</td>
<td>1 (4.4%)</td>
<td>9 (39.1%)</td>
<td>13 (56.5%)</td>
<td></td>
</tr>
</tbody>
</table>
The role of gestational age on newborn screening was also examined. A categorical variable was created for GA which divided patients into two groups: GA less than 37 weeks (preterm) and GA greater than or equal to 37 weeks (full-term). There was no statistically significant association between GA alone and the incidence of false-positives. A stratified analysis was also conducted to examine the relationship between type of nutrition and incidence of false-positives while controlling for the effect of gestational age. A Cochran-Mantel-Haenszel test of general association was significant, indicating that there is an association between type of nutrition and false-positives controlling for gestational age ($p < 0.001$). Fisher’s exact test demonstrated that there was evidence of a relationship between nutrition type and incidence of false-positives for babies born earlier than 37 weeks gestation ($p < 0.01$) whereas there was no significant association for babies born at 37 weeks or later. In preterm infants, 8.11% who received both EN and PN had a false-positive compared to 0% who received EN exclusively. For preterm neonates in the PN only group, 23.53% had a false-positive.

There were 202 patients who received PN (either PN only or PN + EN). Each of these 202 was given parenteral protein and all but 22 also received parenteral lipid. In this group of patients, a chi-square test was performed to see if there was an association between the timing of first administration of parenteral protein and the incidence of false-positives. A categorical variable was created for age at protein initiation with three levels: 0 to less than 12 hours, 12 to less than 24 hours, and greater than or equal to 24 hours. The results of this test were not statistically significant. There was also no significant association between the incidence of false-positives and whether or not patients received parenteral lipids in addition to protein.

The effect of type of enteral nutrition was scrutinized for its role on newborn screening in the group that received both EN and PN ($n=165$). Because of the small number of false-
positives, the EN only group was excluded from this particular analysis. Further, PN had already been shown to increase risk of an abnormal result. The chi-square test was significant demonstrating a relationship between type of EN and outcome of NBS results \((p < 0.001)\). In the group of neonates who received both breast milk and formula, 3.8% had a false-positive compared to 6.1% in the group that was given formula exclusively. However, 30% of the infants who received breast milk as their only source of enteral nutrition had a false-positive. Refer to Table 6 for information about the type of nutrition provided to infants with false-positive screening results.

**Discussion**

The type of nutrition provided to the NICU population was significantly related to the outcome of the newborn screening test. Although the exact risk of false-positive outcomes could not be predicted based on nutrition type alone in this univariate analysis, there seemed to be a trend that supported the hypothesis. Infants who received exclusive PN seemed to be more likely to have a false-positive outcome on NBS results than those who received EN. Unfortunately, the relatively small sample size limited statistical analysis capable of supporting the hypothesis. It was therefore felt that the hypothesis could not be confirmed. However, certain tendencies could be identified; infants who received both parenteral and enteral nutrition seemed less likely to have a false-positive than those with PN only, but more likely than the EN only group.

The Clinical and Laboratory Standards Institute has proposed a standardized screening protocol for preterm and critically ill infants (15). The document included a discussion of factors that can influence NBS results, including PN. Many labs can determine if an abnormal screen is related to PN by analyzing the ratios of the specific amino acids. However, not every false-
positive can be determined in such a manner. The present study agrees that blood sample
collection protocols should consider common NICU occurrences, such as the administration of
PN, in an effort to avoid or minimize false-positive results.

Gestational age, which correlates strongly with birth weight, was not found to be a
significant factor in NBS results in the categorical analysis. However, when nutrition type was
scrutinized based on preterm versus full-term gestational maturity, the results suggested an
interaction between the two on NBS results. Unfortunately, that exact relationship was not
delineated in the present univariate analysis, although some associations can be inferred based on
the percentages. Infants born before 37 weeks gestation who received only PN tended to have a
higher rate of false-positives than other preterm infants who were fed with EN exclusively or EN
plus PN. Such a trend suggests that premature babies do not tolerate PN as well as their
gestationally mature counterparts and agrees with the findings of Clark et al. (19). The authors
hypothesized that high intakes of IV protein can saturate the metabolic pathways in preterm
infants and thus result in excessive amino acid pools. The impact can be seen on newborn
screening blood sample analysis which detects high levels of metabolites, including amino acids.

Determining the exact interaction between nutrition support and GA with regard to
newborn screening will be important as the present findings also conflict with published data.
Thureen et al. found that GA had no effect on PN tolerance (20). Although the sample size was
small (n=19), they concluded that critically ill infants tolerate IV amino acids regardless of
gestational maturity and birth weight. In that study, however, PN tolerance was assessed by
blood levels of creatinine, plasma urea nitrogen, and metabolic acidosis, not NBS results, making
comparison somewhat difficult.
The timing of IV protein administration was not related to the results of newborn screening. The mean age of IV protein initiation was 14.5 hours and 62% were younger than 12 hours. Further, the addition of lipid to the IV nutrition regimen had no impact on the risk of false-positives. These results further validate the findings of te Braake et al. who studied the timing and tolerance of IV nutrition in VLBW infants (21). Their prospective study administered IV AAs within 2 hours after birth and the infants demonstrated satisfactory tolerance as assessed by nitrogen balance, blood gas, and weight measurements. They concluded that VLBW infants can tolerate IV amino acid administration immediately after birth. While effects on newborn screening results were not assessed, the current findings agree with te Braake and other investigations of the acceptable tolerance of early IV nutrition.

The type of EN was scrutinized in the group that received both PN and EN for possible effect on NBS results. The role of EN type could not be appropriately analyzed by including the EN only group in the analysis as the infants who received PN were shown to have a greater risk of a false-positive. Surprisingly, enteral feedings of breast milk exclusively seemed to increase the risk of an abnormal NBS result in this group. Such a finding was certainly unexpected and raises many questions.

Undoubtedly, breast milk is the best choice of EN for any infant. The numerous benefits of it have been clearly and unequivocally ascertained and such is not disputed here. However, there is now the question of why breast milk seems to contribute to the incidence of false-positive NBS results. Perhaps this is best explained by the composition breast milk itself. Preterm breast milk is higher in protein compared to term breast milk (22). It can be inferred that this higher protein concentration may be contributing to abnormal blood metabolite levels detected by newborn screening technologies. Unfortunately, the exact role of gestational
maturity and EN provided could not be assessed for their effect on NBS results in the present univariate analysis. This certainly deserves more scrutiny in the future.

A second explanation of this supposed phenomenon may exist. Breast milk is by far the most easily absorbed form of enteral nutrition for infants. It is possible that the rapid and efficient absorption of breast milk contributes to higher circulating levels of metabolites and therefore a greater risk of false-positive NBS results. If such a finding is validated in future research, perhaps separate cutoff values for abnormal levels of metabolites should be set for critically-ill and preterm newborns who receive breast milk prior to their screen. However, in premature babies, there is a question about the absorptive capacity of their immature gastrointestinal tract. The under-developed digestive systems of this population may negatively affect absorption, making the current results even more puzzling.

One final rationalization of the surprising findings in the present study may lie in the composition of preterm formula as opposed to breast milk. Formula manufacturers have designed and refined products based on years of research. Presumably, this research has resulted in high-quality specialized formulas for the premature infant population.

In the present study, only 1 false-positive came from the exclusive EN group; this infant had received a combination of both breast milk and formula. In order to truly assess the role of type of EN on newborn screening, a larger sample of infants with false-positive results who received EN only would need to be analyzed. Such information would offer more insight into the effect of the 3 possible forms of EN without PN as a confounding factor. Further, it is unknown if the quantity of breast milk and/or formula plays a role in NBS results, as such was not addressed in the present study. Much can certainly be learned from future research in this area.
It would have been the most thorough method to analyze the data, univariate analysis was utilized because the relatively low number of false-positive test results would not support such a complex statistical model with several predictors. In order to conduct the analysis using logistic regression, additional data would be needed. Specifically, a larger overall sample with subsequently matched groups of patients who received either EN or PN exclusively, and a larger sub-group with false-positives, would make regression analysis more useful. Further, it would be helpful to consider other variables that may be associated with the incidence of false-positive screening results such as antibiotic administration, blood transfusions, and quantity of EN and/or PN provided. Finally, the presumed interaction between nutrition, gestational age, and NBS results could be more adequately described in future studies utilizing multiple logistic regression analysis.

Nutrition is certainly not the only factor that can contribute to abnormal NBS results in the preterm and critically ill infant population. However, it clearly has a considerable role. There is much room for improvement in the screening process for this special group of infants to decrease the costs and anxieties associated with false-positives. A standardized screening protocol is undoubtedly needed. The present study suggested a relationship between PN and abnormal NBS results but did not quantify the levels of abnormal metabolites in the blood circulation of these newborns. More research is needed to delineate the contribution of PN to metabolite levels in order to adjust cutoffs for abnormal screening results and thus avoid false-positives. Perhaps there should be separate cutoff levels for NICU infants who received PN prior to their screen and those who did not. The present study also suggests that the timing of IV protein administration does not play a role in abnormal NBS results and thus may not need consideration in future protocol recommendations. The nutrition provided did not correlate with
the categories of disorders flagged and thus may not have a role in predicting false-positive outcomes. Finally, the type of EN provided to the infant seems to have an impact on NBS results while the role of gestational age is not entirely clear. Each of these may deserve consideration on the development of standardized protocols.

Conclusions

Nutrition is clearly one of the factors that can influence newborn screening. Although not statistically supported, the present study has demonstrated that parenteral nutrition contributed to false-positive newborn screening results in preterm and critically ill infants. There is yet much to learn regarding the specific role of enteral nutrition. This work has contributed to the body of knowledge on the impact of nutrition support on newborn screening that will shape future standardized protocols in an effort to reduce false-positives and prevent or minimize unnecessary costs and anxieties.
References


Chapter 4: Conclusions

Summary

The present study found that nutrition provided to preterm and critically ill infants had a significant impact on newborn screening (NBS) results. Specifically, infants who received parenteral nutrition (PN) seemed to be more likely to experience a false-positive screening than those who received enteral nutrition (EN) only, or EN plus PN. The age of initiation of intravenous nutrition had no impact on screening results, suggesting that infants of any gestational maturity tolerate PN. Similarly, the addition of parenteral lipid was not a factor in abnormal results. The type of EN provided seemed to have an impact on screening in a way that was not expected; feedings of breast milk exclusively seemed to increase risk of a false-positive result when compared to formula exclusively or breast milk plus formula (in the group that also received PN). This may be explained by the fact that breast milk is efficiently absorbed and seems to quickly contribute to abnormal blood metabolite levels and thus impact screening results. Gestational age did not in and of itself impact NBS results, but seemed to play a role when the data were stratified by type of nutrition provided. While the exact relationship could not be statistically defined, there was an apparent trend. Preterm infants who received PN were more likely to experience a false-positive than infants who received EN exclusively or EN plus PN. There was not a similar tendency for full-term infants. Finally, the nutrition provided to the infants did not seem to correlate with the type of disorder flagged on the NBS and thus cannot be used to predict false-positive outcomes.
Implications for Research and Practice

One of the limitations of the current investigation was small sample size of specific subgroups, namely false-positives and infants who received either EN or PN exclusively. Development of a multi-center trial could be one avenue in which to achieve the optimal sample size. In addition to increasing sample size, future studies should also consider more variables that are known to impact NBS results in order to achieve a deeper understanding of what combination of treatments may produce abnormal screening results. Such variables that deserve consideration include but are not limited to antibiotic administration, blood transfusions, and quantity of EN and PN provided. The present study suggested the possibility of an interaction between gestational age and type of nutrition provided, but could not conclusively delineate the relationship. It is possible that other interactions exist.

The overall goal of research into the factors that influence NBS is to create a standardized screening protocol for the neonatal intensive care unit (NICU) population that would minimize the incidence of false-positives and thus reduce the cost and anxiety associated with repeated screening and diagnostic work-up. The NICU is of particular interest because of the high rate of false-positive screening results in that population as opposed to the wellborn population. These unique infants inherently demand special consideration in every aspect of their care and newborn screening is no different.

Conclusions

Nutrition is clearly one of the factors that can influence newborn screening. Although not statistically supported, the present study has demonstrated that parenteral nutrition contributed to false-positive newborn screening results in preterm and critically ill infants. There
is yet much to learn regarding the specific role of enteral nutrition. This work has contributed to the body of knowledge on the impact of nutrition support on newborn screening that will shape future standardized protocols in an effort to reduce false-positives and prevent unnecessary costs and anxieties.
## Appendix A: Select Disorders Detectable on Newborn Screening

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<table>
<thead>
<tr>
<th>Disorder</th>
<th>Problem</th>
<th>Natural Course without Adequate Treatment</th>
<th>Optimal Screening Time</th>
<th>Treatment Following Screening</th>
<th>Effectiveness of Screening and Early Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKU</td>
<td>Impaired metabolism of the amino acid phenylalanine</td>
<td>Severe MR, eczema, epilepsy, gait and stance problems, shortened lifespan</td>
<td>3 to 5 days</td>
<td>Phenylalanine-restricted dietary products continued indefinitely</td>
<td>Normal mental and physical development, may have slightly decreased IQ</td>
</tr>
<tr>
<td>CH</td>
<td>Deficiency of the hormone thyroxine needed for brain development and physical growth</td>
<td>MR, physical abnormalities, premature death, abnormal growth</td>
<td>3 to 5 days</td>
<td>Thyroxine supplement indefinitely</td>
<td>Normal mental and physical development</td>
</tr>
<tr>
<td>GA</td>
<td>Deficiency of the enzyme needed to metabolize galactose, a type of sugar in milk</td>
<td>Life threatening septicemia and liver damage in infancy; MR and cataracts in survivors; leads to death if left untreated (mild and asymptomatic forms may occur)</td>
<td>Less than 5 days</td>
<td>Elimination of galactose-containing foods indefinitely</td>
<td>Life saved in neonatal period, low IQ, normal physical development in majority of cases, coordination and speech problems, gonadal failure in some females</td>
</tr>
<tr>
<td>MSUD</td>
<td>Deficiency of the enzyme needed to metabolize branched-chain amino acids</td>
<td>Life threatening academia and neurologic dysfunction in infants, MR in few survivors, leads to death if untreated</td>
<td>1 to 5 days</td>
<td>Dietary restriction of branched-chain amino acids indefinitely</td>
<td>Life saved in neonatal period, normal mental and physical development, risk of sudden death at a later age in event of metabolic intoxication</td>
</tr>
<tr>
<td>HC</td>
<td>Deficiency of the enzyme needed to metabolize homocysteine</td>
<td>Developmental retardation, dislocation of ocular lenses, life-threatening thrombosis, skeletal manifestations</td>
<td>3 to 4 weeks</td>
<td>Dietary restriction of methionine and cystine and vitamin B6 supplementation indefinitely, betaine and folic acid for some</td>
<td>Normal mental development, some physical problems may remain such as lens dislocation and thrombosis</td>
</tr>
<tr>
<td>BD</td>
<td>Deficiency of the enzyme needed to metabolize the B vitamin biotin</td>
<td>Life-threatening neurologic dysfunction, developmental delay, skin findings, and hearing loss in survivors (mild and asymptomatic cases may occur)</td>
<td>Less than 5 days</td>
<td>Oral biotin supplements</td>
<td>Life saved in neonatal period for some, avoidance of neurologic damage</td>
</tr>
<tr>
<td>Condition</td>
<td>Description</td>
<td>Symptoms</td>
<td>Prophylaxis</td>
<td>Outcome</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>-------------</td>
<td>----------</td>
<td>-------------</td>
<td>---------</td>
<td></td>
</tr>
<tr>
<td>SCA</td>
<td>Abnormality of the red blood cells that causes them to be sickle shaped</td>
<td>Life-threatening infections, especially up to 3 years of age, chronic hemolytic anemia and vaso-occlusive crises</td>
<td>First week</td>
<td>Prophylactic penicillin and pneumococcal vaccine, ongoing supportive therapy</td>
<td>Reduction of risk of death in infancy and early childhood from complication of infection</td>
</tr>
<tr>
<td>CAH</td>
<td>Inability to produce the hormones needed to manage stress and control salt content, excessive buildup of male hormones</td>
<td>Life-threatening salt wasting crises for some in infancy, reproductive dysfunction, abnormal physical development</td>
<td>2 to 5 days</td>
<td>Intravenous salt solution, hormone therapy</td>
<td>Life saved in neonatal period for some, aids sex assignment in infant girls, normal sexual development</td>
</tr>
</tbody>
</table>

Source:

Abbreviations: PKU = Phenylketonuria; MR = Mental Retardation; CH = Congenital hypothyroidism; GA = Galactosemia; MSUD = Maple Syrup Urine Disease; HC = Homocysteinuria; BD = Biotinidase Deficiency; SCA = Sickle Cell Anemia; CAH = Congenital Adrenal Hyperplasia
Appendix B: Data Entry Fields for SoftMed

MRN
Birth Date
Birth Time
Gestational Age (weeks)
Birth Weight (grams)
Type of Nutrition
   E = Enteral
   P = Parenteral
   B = Both
If Enteral, Type
   B = Breast milk
   F = Formula
   H = Human milk fortifier
   C = Breast milk + formula
If Parenteral:
   Glucose?
   Y = Yes
   N = No
      If Yes Glucose
         Date:
         Time:
   Lipid?
   Y = Yes
   N = No
      If Yes Lipid
         Date:
         Time:
   Protein?
   Y = Yes
   N = No
      If Yes Protein
         Date:
         Time:
Nutrition Start (hours after birth)
Date of NBS Collection
Time of NBS Collection
NBS Result
   N = Normal
   A = Abnormal
Type of Disorder Flagged
   N = N/A
   A = Amino Acid Disorder
   O = Organic Acid Disorder
Fatty Acid Oxidation Disorder
Galactosemia

Repeat Screen Results
N = normal
A = abnormal

Type of Repeat Screen Disorder Flagged
N = N/A
A = Amino Acid Disorder
O = Organic Acid Disorder
F = Fatty Acid Oxidation Disorder
G = Galactosemia

False Positive Initial Screen?
Y = Yes
N = No

IEM Diagnosis (if applicable)
0 = N/A
1 = PKU
2 = HCY
3 = MSUD
4 = CIT
5 = ASA
6 = TYR I
7 = IVA
8 = GA I
9 = HMG
10 = MCD
11 = MUT
12 = Cbl A,B
13 = 3MCC
14 = PROP
15 = BKT
16 = MCAD
17 = VLCAD
18 = LCHAD
19 = TFP
20 = CUD
21 = GALT

Type of IEM Diagnosis (if applicable)
N = N/A
A = Amino Acid Disorder if “IEM Diagnosis” = 1,2,3,4,5, or 6
O = Organic Acid Disorder if “IEM Diagnosis” = 7,8,9,10,11,12,13,14, or 15
F = Fatty Acid Oxidation Disorder if “IEM Diagnosis” = 16,17,18,19, or 20
G = Galactosemia if “IEM Diagnosis” = 21
Appendix C: IRB Approval Letter

September 15, 2009

Brittany Cochran, RD
75 Prominence Ln Apt 205
Christiansburg, VA 24073

re: Nutrition Support and Newborn Screening in the NICU Population: Is There a Link?

Dear Ms. Cochran:

I am pleased to inform you that the Carilion Institutional Review Board (IRB) has reviewed the above-mentioned protocol in an expedited manner according to 45 CFR 46.110 and 21 CFR 56.110. The research project was determined to present no more than minimal risk to human subjects and was found to have appropriate protections so that risks related to breach of confidentiality are no more than minimal. This research project met the expedited criteria outlined in 63 FR 60364-60367 category (5) Research involving materials (data, documents, records, or specimens) that have been collected, or will be collected solely for non-research purposes (such as medical treatment or diagnosis).

According to 45 CFR 46.111, the following requirements were satisfied in order for approval to be granted:

- risks were minimized;
- risks to subjects are reasonable in relation to anticipated benefits, if any, to the subjects, and to the importance of the knowledge that may reasonably result from the study;
- selection of the subjects was equitable given the purpose of the research;
- informed consent will be sought from and documented for each prospective subject unless the conditions for a waiver of documentation for consent were met;
- when appropriate, the research plan makes adequate provisions for monitoring the data collected to ensure safety of subjects; and
- adequate provisions to protect the privacy of the subjects and to maintain the confidentiality of the data were made.

The Carillon IRB has reviewed this protocol according to 45 CFR Subpart D—Additional Protections for Children Involved in Research. The research project has met the criteria for approval under §46.404 - Research not involving greater than minimal risk. The Carillon IRB finds that no greater than minimal risk to children is presented and that adequate steps have been taken to protect the privacy and confidentiality of the subjects.

The Carillon IRB has waived the requirement of parental permission and assent by children as outlined in 45 CFR 46.116(d), as it finds that the research involves no more than minimal risk to subjects, the waiver will not adversely affect the rights and welfare of subjects, and the research could not practically be carried out without the waiver, and whenever appropriate, the subjects will be provided with additional pertinent information after participation.

Institutional Review Board
2001 Crystal Spring Avenue, SW, Suite 202 Roanoke, VA 24014-2465
540-853-0728 p 540-983-5323 f
The Carillon IRB has granted a Waiver of Authorization to use and access protected health information for the above-mentioned study. The IRB has determined that the Waiver of Authorization satisfies the following criteria outlined in 45 CFR 164.512(i)(2)(A):

- The use or disclosure of protected health information involves no more than minimal risk to the privacy of individuals, based on the presence of the following elements:
  - An adequate plan to protect the identifiers from improper use and disclosure
  - An adequate plan to destroy the identifiers at the earliest opportunity consistent with conduct of the research, and
  - Adequate written assurances that the protected health information will not be reused or disclosed to any other person or entity, except as required by law, for authorized oversight of the research study, or for other research for which the use or disclosure of protected health information would be permitted by this subpart
- The research could not practically be conducted without the waiver or alteration; and
- The research could not practically be conducted without access to and use of protected health information

The waiver of authorization has been reviewed and approved under expedited review procedures and applies only to the data outlined in your application/protocol.

Approval to the study is granted for a period of twelve months, effective today. Approval of your research by the Carillon IRB provides the appropriate review as required by federal and state laws governing human subjects' research. This letter conveys IRB approval only and does not grant institutional approval. If your research involves any Carillon facilities, then separate arrangements must be made with the appropriate hospital or medical staff, departments or committees.

Additionally, the following documentation must be provided to the Carillon IRB:

- Continuing Review Application 30 days prior to the expiration date, providing a summary of the project to date and requesting continuation of the original project. If the original project is discontinued the IRB must be notified within seven business days.
- Serious adverse events and unanticipated problems that are unexpected and related, as outlined in the IRB Guidelines, within seven business days of the investigator becoming aware of them.
- Copies of reports from Data Monitoring Committees or auditing/monitoring reports from a sponsor are to be sent to the IRB Research Compliance Officer within seven business days.
- Any unplanned protocol variance that could adversely affect the safety or welfare of subjects, or the integrity of the research data, within ten days of becoming aware of the variance. Other unplanned variances may be recorded on a log and submitted with continuing review reports. Any changes to the research study must receive IRB approval before those changes can be implemented unless subject safety is directly affected.

Also, please find attached a form titled, "Carillon Clinic IRB Research Organization Checklist.” The IRB is distributing this tool to provide guidance on maintaining research documentation for investigator-initiated studies.
The Carilion IRB would like to thank you for allowing us the opportunity to review this protocol. We look forward to learning of your results.

Sincerely,

Charles A. Hite, MA, CIP
Human Protections Administrator

cc: Daniel Harrington, MD, VP, Academic Affairs
    Nancy Misicko, MD, Chair, Carilion IRB
    Carrie Boyd, Administrative Director, Office of Sponsored Projects
    Judie Snipes, Privacy Officer
    Barbara Winfield, Manager, HIM
    Mattie Tenzer, Director, Clinical Integration and Analytics
    Alice Ackerman, MD, Chair, Dept. of Pediatrics
    IRB files
Appendix D: Permission for Table Reproduction
“Select Disorders Detectable on Newborn Screening” in Appendix A

Hi Brittany,

Thank you for your request. Indeed, I do grant you permission to reproduce this table. I believe this table was adapted from another table; if so, probably best to cite the original source as well...

Katy

Please note that my email address has changed to: meilleurk@mail.nih.gov

Hi Ms. Balk. I am currently a Master's candidate in the Human Nutrition, Foods, and Exercise program at Virginia Tech. Soon I will be starting my research looking into the potential association between nutrition support and newborn screening results in the NICU population. I have included two of your articles in my literature review and have found them to be very helpful. I am writing for permission to reproduce a table from your article in my thesis. It is "Table 1: Select Disorders Detectable on Newborn Screening" found in your 2007 article "Recommended Newborn Screening Policy Change for the NICU Infant" published in Policy, Politics, and Nursing Practice. Your permission to use this table would be greatly appreciated! Thank you for your time.

Brittany Cochran
Registered Dietitian
Master's Candidate-Virginia Tech
bcochran@vt.edu