The Practitioner’s Role in Assuring Timely Diagnosis & Referral

The Nebraska Newborn Screening Program continually works to assure rapid turn around times when it comes to identifying the at-risk newborns who need further confirmatory testing, diagnosis and intervention or treatment services. We all want the best outcomes for babies affected with the screened conditions in order to prevent serious morbidity and infant mortality.

This issue focuses on new population challenges facing practitioners when screening for hemoglobinopathies and on new guidelines for the diagnosis, treatment and management of cystic fibrosis.

“In newborn screening, many of the conditions we screen for, can present along a spectrum from mild to severe. Getting to the correct diagnosis can be challenging in some cases. It is becoming increasingly important to rapidly consult with and/or refer to pediatric subspecialists to help sort out diagnoses and co-manage patients with screened conditions.”

Julie Luedtke
Program Manager

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- New Conditions to be Added to the Screening Panel
Why an update?

Newborn screening (NBS) for Cystic Fibrosis (CF) began in Nebraska in January of 2006. The results of this screening are that:

- the vast majority who have CF are detected in the newborn period,
- babies with CF diagnosed because of screening are much healthier at the time of diagnosis than when they were detected because of symptoms and they have avoided the “diagnostic odyssey”,
- some of the carriers of a CF causing mutation are identified and the families are able to receive genetic counseling,
- some babies are identified through the screening that do not receive a definitive diagnosis but are then followed for symptom development and are said to have Cystic Fibrosis Related Metabolic Syndrome (CRMS),
- from 2006 until 2017 through NBS 86 babies were diagnosed with CF, 135 babies were identified as carriers of a CF mutation, 14 babies were diagnosed with CRMS.
- False Negatives - Six children were found to have CF not detected by screening (all had IRT values below the cutoff, some were very low). These children serve as a reminder that the NBS for CF cannot find all of the children with CF. Any baby or child with symptoms of CF should be promptly evaluated even if the screen was WNL.

All 50 states have been screening for CF since 2010. Many other countries also screen their newborns for CF. As a result of NBS the diagnosis of CF now occurs at an earlier age and often before any detected symptoms. This change in age at time of diagnosis provides the opportunity for earlier treatment. Since most CF patients in the US are now diagnosed through NBS there is greater experience with early intervention. Yet the screening of newborns for CF has also resulted in the identification of babies with abnormal screens who do not meet the criteria for a diagnosis of CF but cannot have the diagnosis ruled out either. They may have equivocal sweat test results and/or poorly understood mutations in the Cystic Fibrosis Transmembrane Regulator (CFTR) gene.

Timeliness of the NBS process has been of recent concern in the US. With the shift to NBS as the method of identification of most CF patients, there has also been greater scrutiny of the processes between receipt of NBS results and the diagnosis of CF. The accumulated experience, research and questions prompted the Cystic Fibrosis Foundation to address the current situations with the issuance of new guidelines for the diagnosis of CF.

New Cystic Fibrosis Foundation Guidelines for the Diagnosis of CF

The Cystic Fibrosis Foundation (CFF) undertook a consensus process including experts from 9 different countries. Their goal was to update the diagnostic guidelines and to attempt to standardize diagnostic terminology and definitions. The guidelines address both the situations where a diagnosis of CF is clear and the complicated situations when a baby cannot be given a diagnosis of CF but has testing results that do not promptly rule out CF. While this update cannot cover all of the information and recommendations provided in the guidelines it will attempt to summarize some of the key information.
The “Cystic Fibrosis Foundation Consensus Guidelines for Diagnosis of Cystic Fibrosis” were published as a supplement to the February 2017 issue of the Journal of Pediatrics, Volume 181, Supplement, S1-S58.

It’s all about the sweat…

The new guidelines for the diagnosis of CF emphasize the necessity of a functional test to confirm the diagnosis of CF. In almost all cases this will be a sweat test which is considered the “Gold Standard” when diagnosing CF. In Nebraska it is advised that the sweat test be done at an Accredited CF Center in order to ensure that the testing is performed in accordance with established protocols.

Newborns can be sweat tested as early as 48 hours of age. However, in order to avoid inadequate specimens, it is recommended that babies are > 2 kg and have a corrected gestational age of 36 weeks or greater when they are tested.

Nebraska’s screening lab PerkinElmer Genetics, reports results as positive, inconclusive or WNL. For the purpose of this update (to be consistent with the guidelines) a positive NBS result implies a positive result or an inconclusive result when a sweat test and referral are recommended.

If a baby has a positive NBS result, a family history of CF, or clinical symptoms suggestive of CF, a sweat chloride result of ≥ 60 mmol/L confirms a diagnosis of CF. In a similar situation a sweat chloride test result of 30-59 mmol/L is considered intermediate and indicates the baby may have CF. Further work up may be needed for a diagnosis. If the baby is known to have 2 CF-causing mutations on separate chromosomes a sweat test result of ≥ 30 mmol/L is also considered confirmatory. A result of < 30 mmol/L makes a diagnosis of CF unlikely. The cut off at 30 mmol/L previously applied to babies less than 6 months of age but has now has been changed to apply to all sweat test results. Sweat tests may need to be repeated when results are intermediate and/or symptoms of CF are present.

The guidelines discuss interpretation of sweat test results in more detail. Other tests to measure CFTR dysfunction include nasal potential difference (NPD) and intestinal current measurement (ICM). However, at this time, these tests are not widely available and further validation of their results are ongoing.

and the DNA…

Testing for mutations of the CFTR gene contributes to the process of diagnosing CF. Mutations may be discovered on the panel of mutations included in the NBS. However, the NE NBS extended panel of mutations includes only 39 specific mutations. There have been more than 2000 mutations of the CFTR gene discovered. Further genetic testing may be required to determine if other mutations are present.

The guidelines suggest utilizing the Clinical and Functional Translation of CFTR (CFTR2) database to aid in the understanding of the effect of the presence of specific CFTR mutations. CFTR2 categorizes mutations as:

- CF-causing
- varying clinical significance
- unknown clinical significance
- non-CF-causing

Two CF-causing mutations on different chromosomes and a sweat test result of > 30mmol/L is considered confirmatory for CF. A mutation of varying clinical significance along with a CF-causing mutation or another mutation of varying clinical significance may result in CF. Unknown significance implies that the mutation has not yet been fully characterized by CFTR2 and may fall into any of the other three categories. Those mutations that are non-CF-causing are not expected to cause CF even in the presence of another CF-causing mutation.
The lack of two CF-causing mutations does not rule out the possibility of CF. At this time there is an ongoing effort to discover and classify mutations of the CFTR gene and additions will be made to CFTR2. There is also research being done to discover modifiers that affect the various phenotypes seen with the same genotypes. The importance of identifying the mutations present will only increase as new therapies for specific defects caused by CFTR mutations are developed.

**The diagnosis is CRMS/CFSPID. What does that mean?**

In the US, “Cystic Fibrosis Related Metabolic Syndrome” or CRMS is the diagnosis used for situations where there is a positive newborn screen but diagnostic testing is inconclusive. These babies do not have sufficient genetic or sweat chloride results to make a diagnosis of CF. In other countries, Cystic Fibrosis Screen Positive, Inconclusive Diagnosis or CFSPID is the term used to describe the situation. (CRMS will continue to be used in the US in part due to current coding systems.)

This situation is not unusual. Most of these babies do not go on to develop disease early in life. This diagnosis can be difficult to explain to families. Since most studies have only been able to describe short term outcomes, long term outcomes are yet unknown. Some of these babies will develop symptoms of CF and will receive a CF diagnosis.

**Why do we need to hurry?**

When newborn screening for cystic fibrosis first began it was felt that there was more time to deal with the results than there was for some of the other diseases found by newborn screens. While that is true, experience has now shown that there needs to be a greater urgency for earlier diagnosis and treatment than was previously appreciated.

The benefit to screening for CF is achieved through early detection and treatment. The guidelines now state that if babies have a presumptive diagnosis of CF, treatment should not be withheld until a definite diagnosis is achieved.

Improved outcomes are a result of early interventions which help babies maximize their growth potential. Early intervention may also help to avoid situations that now have been observed within the first few weeks of life such as hyponatremic dehydration, malnutrition and infections.

Therefore it is important to expedite evaluation of NBS positive babies at the CF center or by CF physicians if babies are still in the hospital. The premature babies that are too small to have sweat tests, should still be seen by a CF physician in order to facilitate initiation of therapy and/or to advise on appropriate testing to help with the attempts at diagnosis of CF. Having families meet with the CF team begins the process of education and can help with any necessary genetic counseling.

The Nebraska Newborn Screening Program emphasizes the importance of appropriate collection of all NBSs and timely follow up whenever indicated. The new CFF guidelines and input from the Nebraska Regional Cystic Fibrosis Center staff have helped to reiterate the need for prompt attention to babies with positive CF results. Early diagnosis and initiation of treatment for all babies who have an abnormal screen for CF is the shared goal of all involved.
Hemoglobinopathies
Management of Newborns with Abnormal Screen Results
James L Harper MD, Karen Eveans MD, Julie Luedtke

Nebraska has a universal newborn screening system. As such all infants born in NE will be screened. This results in a number of screening results that require confirmation and further investigation.

To screen for hemoglobinopathies, the current system utilizes a batched isoelectric focusing (IEF) analysis. The analysis reports out hemoglobin types by their appearance on an electrophoretic gel. The typical full term neonate has a pattern of fetal and adult hemoglobin. Having only started to make adult hemoglobin a few weeks before birth, the typical pattern seen is “FA”, where F is listed first as it is the largest part of the hemoglobin mixture. The hemoglobin types are listed in descending order of concentration.

The newborn with a normal result needs no further testing. All babies with an abnormal screen result need to have confirmatory testing per the state’s recommendations that comes with your patient’s results. This should be drawn in the newborn period and sent to an appropriate reference lab. For clinically significant results this should be accomplished without delay. In Nebraska, Regional Pathology Services at Nebraska Medicine offers High Performance Liquid Chromatography (HPLC) for quantitation as well as hemoglobin electrophoresis isoelectric focusing (IEF) and supplies an interpretation of the results.

Targeted Conditions
Respond Immediately

FS is the pattern typically seen in newborns with sickle cell disease. This pattern implies the presence of fetal and sickle hemoglobins only. The test results should be confirmed and immediate referral to a pediatric hematologist should be made. Genetic testing may have a role in the evaluation of this pattern as it may represent either Hemoglobin S-S disease or Hemoglobin S/Beta (0) Thalassemia. PerkinElmer Genetics, the lab used by Nebraska for newborn screening, sometimes reports FS/Beta Thalassemia when a beta thalassemia mutation is identified. The panel of mutations used as part of screening is very small and does not detect all of the possible beta thalassemia mutations. The difference between S-S disease and S/Beta thalassemia may be detectable by a CBC later in infancy and as it does not change the treatment, genetic testing is not routinely performed.

FSA is the pattern that can indicate Hemoglobin S/Beta (+) Thalassemia. This result implies decreased production of hemoglobin A due to a beta thalassemia mutation that has a milder affect than a Beta (0) thalassemia mutation that causes no production of hemoglobin A. Confirmation should not be delayed. Both IEF and HPLC should be used for confirmation and the baby referred to a pediatric hematologist.

FSC is the pattern that suggests Hemoglobin S-C Disease. This condition also needs confirmation and rapid referral to a pediatric hematologist. Patients can vary widely in their clinical presentations.

Multiethnic families may have family members with other sickle cell syndromes (such as Hemoglobin S-D disease or Hemoglobin S-E disease). Results such as FSD or FSE are true disease states and should be approached as such. These conditions are similar to Hemoglobin S-C Disease and require confirmation and referral to a pediatric hematologist.
Management of these infants should be a combination of primary care and pediatric hematology in a medical home model. Current guidelines from the National Heart Lung and Blood institute are available at https://www.nhlbi.gov/sites/www.nhlbi.nih.gov/files/sickle-cell-disease-report.pdf

Penicillin VK prophylaxis should be started on all infants with a sickle cell syndrome (FS, FSA, FSC, FSD and FSE) before two months of age. Duration of prophylactic penicillin is variable (depending on the patient’s disease state and his or her infection and vaccine history) but generally may be stopped at six years of age.

**F only** is the test result that is classically associated with severe forms of Beta Thalassemia Major in which no hemoglobin A is produced. The result should be confirmed immediately. Confirmatory testing and referral to a pediatric hematologist should occur immediately. Patients with this condition may require lifelong transfusions.

**FE** is a test result that would be seen in Hemoglobin E Disease and Hemoglobin E/Beta (0) Thalassemia. Differentiation between these two conditions is extremely important because Hemoglobin E Disease generally does not cause significant symptoms but Hemoglobin E/Beta (0) Thalassemia is more variable with the most severely affected requiring lifelong transfusions. Confirmation should be done without delay and referral to a pediatric hematologist may be necessary to help with precise diagnosis.

As the Population Changes Screening Results May Change

**Example: Beta Thalassemias**

Beta thalassemia traits are not usually detected on newborn screens unless they are coexistent with other hemoglobinopathies. In Nebraska when a result is suspicious for a coexistent beta thalassemia (results FSA, FCA, etc.) or beta thalassemia major (result F only) the lab reflexes to a test for certain mutations that cause beta thalassemia.

Immigration trends into Nebraska have affected the newborn screening system for hemoglobinopathies. The majority of immigrants into Nebraska in 2015 were from Latin America (70,599) but the second biggest group came from Asia (35,974). The newborn screening lab has built in reflex genetic testing for certain subtypes of beta thalassemias. Their choice of mutations to test for was based upon historical genetic data of the ethnic populations in the US. These detect a significant fraction of beta thalassemia variants arising in the Sicilian, Greek, and African regions. However, they are not detecting the common variants from the Arab regions or in the Indian or Asian populations. While it is possible that an infant with these ancestries could be detected by the screening system, it is highly likely that their beta thalassemia subtype will not be identified. Therefore, clinical judgment regarding formal gene sequencing and deletion/duplication analysis is required.

**Example:**

**Sickle Cell Traits**

In 2015 in Nebraska, Africans were the third largest group of immigrants with 11,981. It is important to realize that this immigration has brought with it more people at risk for hemoglobinopathies, but also more variance in risk. Classically, we have learned that the sickle cell trait carriage rate in the US is between 8 and 10% of the African American population. In Ghana, the carriage rate of sickle cell trait is 30% with 2% of all babies born there having sickle cell disease. In other nations where immigrants to Nebraska are from, the carriage rate can vary widely. For example, India's population varies from 1% to 40% sickle cell trait incidence. Given this, it is important to seek early consultation and genetic testing which may play a larger role in the confirmation and evaluation of these infants in future years.
Other Conditions
Still need follow up

FA + Bart’s is indicative of Alpha Thalassemia, a condition of decreased alpha globin production. Bart’s Hemoglobin is the product of unbalanced alpha and gamma globin production resulting in more gamma globin proteins than alpha globin proteins being produced by the fetus. The excess gamma globin proteins condense into a tetramer of gamma globins called Hemoglobin Bart’s. The amount of the imbalance depends on the number of missing genes and/or the degree of dysfunction of mutated alpha globin genes. The primary task for the newborn’s care team is to determine the extent of the alpha gene deficiency. This can be done by quantification of the Bart’s hemoglobin (by HPLC testing) which is higher with 3 gene abnormalities than it is for 1 or 2 gene abnormalities. Hemoglobin H disease (3 gene abnormalities) is commonly associated with very high levels of Bart’s hemoglobin. If Hemoglobin H disease is detected a pediatric hematology consult is needed. A CBC and ferritin level at 6-9 months of age may help to distinguish between 1 or 2 gene involvement.

Alpha thalassemia is concentrated in people of African and south-east Asian ancestry. In Africa, the most common form of genetic damage is a large-loop deletion of one or two of the four alpha globin genes. This is generally a benign condition of the trait form and not of the severity of Hemoglobin H or the most severe, alpha thalassemia major (4 affected genes). Typically for the trait form, family counseling with a genetic counselor or pediatric hematologist will suffice.

In newborns of south-east Asian ancestry, diagnosis can be more complicated as both large loop deletions and missense mutations that result in decreased gene function are seen. In addition to the typical confirmatory testing, genetic analysis is frequently required. Genetic testing can be arranged with a pediatric hematology or genetics consult. The test ordered is called alpha globin sequencing and deletion/duplication analysis. Examples of findings in this population include Alpha Thalassemia (South East Asian) and Hemoglobin Constant Spring amongst others. If the confirmatory study suggests one of these, referral to pediatric hematology is advised.

Increasing immigration from China and southern Asia into Nebraska is creating a situation where these subtypes of alpha thalassemia are increasingly common. Bart’s hemoglobin was present in 122 of the 450 babies with abnormal hemoglobins detected in 2016. In multiethnic families, the combinations of hemoglobin abnormalities may be an additional concern. The family may have no familiarity with hemoglobinopathies or may have had very severely affected children in past generations. Genetic analysis as described is helpful in guiding these families.

FAS is the pattern that suggests sickle cell trait. This is a largely benign condition that should be confirmed. In older patients, under conditions of extreme physical activity, rare cases of rhabdomyolysis and sudden death have occurred. As with all abnormal hemoglobin results, parents should be counseled regarding the hemoglobinopathy and its inheritance pattern and offered genetic testing to determine risks.

FAC is the pattern typically seen in Hemoglobin C trait. Like Sickle cell trait, this is a rather benign condition that is manifested by lifelong red blood cell anomalies (microcytosis and occasional target cells). These patients may at times have mild anemia. Once the diagnosis is confirmed, referral to a pediatric hematologist is not usually required.

FC is the pattern seen in either homozygous Hemoglobin C disease or the double heterozygous condition Hemoglobin C/Beta Thalassemia Syndrome. Hemoglobin C disease is largely benign, manifesting as mild to moderate anemia and microcytosis. To differentiate between the two conditions, genetic analysis is
performed (beta globin sequencing and deletion/duplication analysis). Genetic analysis is typically done only on patients who are manifesting atypical clinical features (severe anemia).

**FAV** is a screening result implying the presence of a rare hemoglobin type. “V” stands for variant hemoglobin. FAV implies that there is **fetal and adult hemoglobin as well as the variant**. Confirmation testing will often reveal the particular abnormality. Many of these are harmless variants, occasionally causing laboratory errors such as errors in measurements of Hemoglobin A1c. Confirmation of these anomalies in the primary family members may be useful given their health history.

**FV** implies the presence of **fetal and variant hemoglobin only** and should be confirmed. If still present on confirmation study, pediatric hematology referral is warranted. Depending on the nature of the variant hemoglobin present, this may have significant clinical impact.

**FAD, FAE, and FAOArab** indicate the heterozygous states of **Hemoglobin D Trait, Hemoglobin E Trait** and **Hemoglobin OArab Trait**. These are largely benign conditions that should be confirmed. Parents should be counseled regarding the hemoglobinopathy, its inheritance pattern and future risks.

**Always Remember…**

Results on a newborn screen must always be interpreted with a baby’s clinical situation considered. These are screening tests. A diagnosis should not be made based on screening results alone. A diagnosis should not be ruled out based upon screening results. By its nature, screening will pick up most of the babies at risk for the conditions but certainly not all of the babies at risk.

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**BIG CHANGES COMING IN 2018**

The 2017 Legislature passed LB 91, and it was signed into law requiring newborn screening for three new disorders.

Pompe Disease and MPS-I (Mucopolysaccharidosis type I), (Lysosomal Storage Disorders) and X-linked Adrenoleukodystrophy will be added beginning July 1, 2018.

The Newborn Screening Program Staff, laboratory experts, pediatric subspecialists and advisors will be preparing screening, follow-up and referral algorithms and recommendations, tracking mechanisms, and parent and professional education to help all involved be prepared for the new screening.

Watch for upcoming newsletters and presentations.
Obstetricians trying to help

In May the Newborn Screening Program distributed laminated posters to all OB providers requesting they post these in their clinics.

The posters are intended to spur expecting women to sign up with a pediatric care provider for their child before he or she is born. It also encourages them to let the birth facility know who their post-discharge provider will be.

In addition to the obvious benefits for newborn and well-baby care, the intent is to increase the number of babies who have a post-discharge provider identified to newborn screening, for quicker notification of any test result needing follow-up testing. In the first weeks of distribution requests for an additional 42 posters have been received.

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Newborn Screening, Saving Lives

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or order online at  http://dhhs.ne.gov/publichealth/Pages/OrderLiterature.aspx

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