NeoReviews™
AN OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF THE PEDIATRICS

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Neoreviews 2000;1;e110
DOI: 10.1542/neo.1-6-e110

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OBJECTIVES

After completing this article, readers should be able to:

1. **Delineate situations in which blood specimens for screening may be overlooked.**
2. **Describe the patterns of thyroid-stimulating hormone and thyroxine concentrations in very low-birthweight infants and the implications of these patterns for thyroid screening programs.**

Prologue

For those of us involved in newborn screening, there is nothing more devastating than learning that the program did not detect an infant who has congenital hypothyroidism. After a moment of disbelief, we ask, "How did this happen and what should or could have been done to prevent such tragedy?" Moreover, in the typical scenario the misfortune frequently is compounded by failure of the clinician to make the clinical diagnosis in a timely fashion. Although these events are rare (probably less than one birth in 1 million), they should not be taken lightly. For the pediatrician, knowing where potential pitfalls lie might spare some future family from the needless anguish of a missed diagnosis.

Introduction

Slightly more than one quarter of a century ago a report emanating from Canada described a procedure for the measurement of thyroxine (T4) in dried blood on filter paper, opening the way for early detection and treatment of infants who have congenital hypothyroidism. Within a relatively short period of time, newborn screening programs for congenital hypothyroidism emerged in most of the industrialized nations, thereby relegating a major scourge of society to the status of a medical curiosity.

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At the outset, most screening programs were relatively primitive, lacking sophisticated data management systems and beset with technical and logistical problems. However, the manufacture of reliable assay materials and the introduction of automation coupled with online data reduction eventually improved these programs. Such improvements, together with growing experience in interpretating test results, overcame many of the drawbacks associated with early screening programs. Although most (but not all) of the shortcomings have been resolved, others have evolved with the passage of time.

Interestingly, most European programs have not been burdened with many of the problems that plague the majority of screening programs in North America. The likely explanation for this can be traced to the different initial approaches to screening, which largely were fashioned by the instability and high cost of commercially prepared radioactive thyrotropin used in the radioimmunoassay (RIA) of thyroid-stimulating hormone (TSH). These disadvantages, combined with the labor intensity of the RIA for TSH, prompted North American programs to employ the less expensive and more reliable tracer T4 as the primary marker. Specimens below an established reference range for normal newborn T4 values then were assayed for their TSH concentrations. In contrast, most European programs, with smaller workloads, used TSH as the primary marker, measuring T4 only in specimens with elevated levels of TSH. TSH as the primary marker is unable to identify conditions such as: 1) secondary or tertiary hypothyroidism, 2) thyroxine-binding globulin (TBG) deficiency, or 3) delayed TSH elevations, which underlie some of the pitfalls of screening. Further advantages and disadvantages of using either TSH or T4 as the initial marker with which to screen will become evident in this discussion.

There are potential pitfalls at every step of the screening process, beginning with the collection of blood in the newborn nursery and ending in the physician's office. Therefore, for purposes of this review, pitfalls have been divided among those associated primarily with the hospital (or birthing center), the screening laboratory, or the physician (Figure).

Pitfalls in the Hospital

FAILURE TO OBTAIN THE BLOOD SPECIMEN

Failure to obtain a newborn blood specimen should be of great concern not only to personnel in screening programs, but to all physicians because of the relative frequency with which it occurs. The magnitude of the problem can be appreciated by the findings of an unpublished study performed in New England several years ago. Omissions in blood collections were tracked by cross-checking birth records against newborn blood specimens received from participating hospitals. Although there was some variation among hospitals, the results indicated that approximately 1% of infants born during a defined interval had no blood specimens available for testing.

ABBREVIATIONS

RIA:	radioimmunoassay
TBG:	thyroxine-binding globulin
T4:	thyroxine
TSH:	thyroid-stimulating hormone

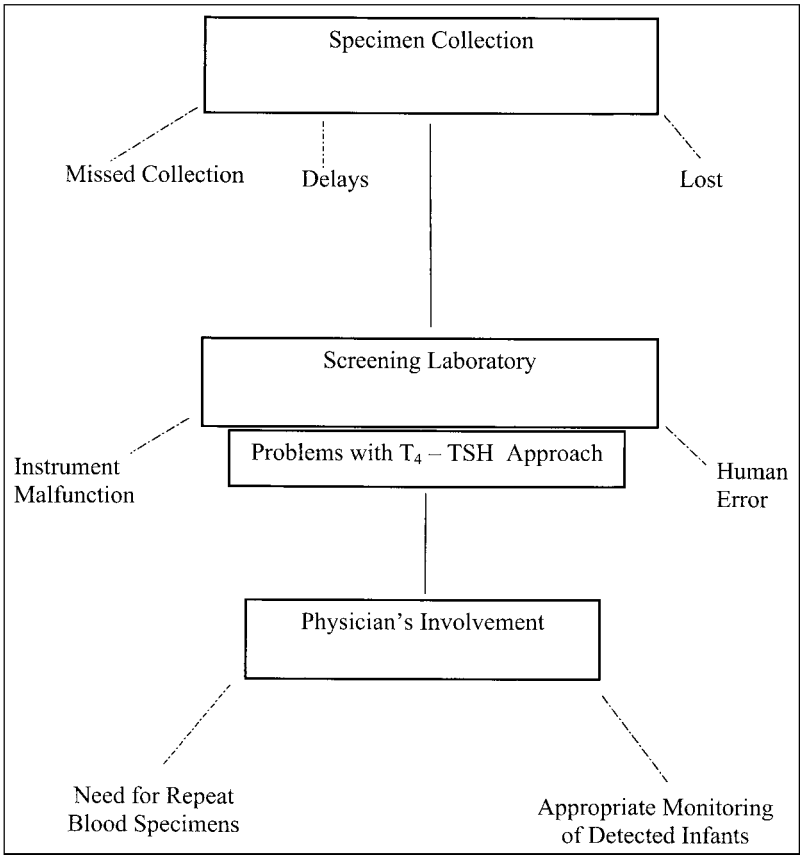

FIGURE. Shortcomings in screening programs for congenital hypothyroidism.

Most of the missed specimens resulted from system failures. The most frequent causes of lapses in blood collection found by the New England program were: 1) early discharge (within 6 to 12 h), typically from a birthing center and less commonly from a hospital, in which a blood specimen is not obtained at the time of discharge and later collection at the pediatrician's office is missed; 2) transfer of an infant to a tertiary care facility for special care, with each institution assuming the other is responsible for the collection; 3) repeated transfer of an infant between the ward and special care within the same hospital, resulting in the overlooked blood specimen; and 4) home births in which the parents either refuse to have the infant screened or neglect to follow recommendations for the collection of the specimen.

Some of these actual occurrences resulted in legal action. One recently settled case involved an infant who was transferred from one hospital to another on the first day of life. A blood specimen was not obtained because both hospitals mistakenly assumed that the other had done so. The diagnosis of congenital hypothyroidism was made several months after birth during which time the clinical stigmata had appeared. Although several specialists were consulted, they failed to arrive at the correct diagnosis because they assumed that the infant had been screened and results were normal.

Some steps can be taken to minimize the occurrence of such pitfalls:

- Institutions should make arrangements for infants who are released before 24 hours of life to have specimens obtained at home within 24 to 48 hours following discharge.
- One person in the nursery should be responsible for maintaining the log in which the date and time of the blood collection is recorded.
- Outreach educational programs that highlight possible pitfalls and their remedies should be available to hospitals, birthing centers, midwives, and other providers.
- On-site help from a representative of the screening program can target institutions that need more monitoring (ie, those that have the poorest record of compliance with specimen collection).

LOST OR DELAYED SPECIMENS

Once a nurse or phlebotomist has obtained a specimen, it is air-dried and placed in an envelope. Generally it is delivered to the screening laboratory via the post office, hospital courier, or local commercial courier. The list of causes for lost or delayed specimens in transport are endless, ranging from a specimen falling behind furniture in the nursery to specimens vanishing between the nursery and the hospital mailroom or during delivery by the hospital courier to the screening laboratory. Despite the best of intentions, systems can and do occasionally fail.

Although there is no guarantee that any system is error-free, the approach used by the New England Program has been reasonably successful. A commercial courier, using uniformed personnel, collects specimens daily at specified times directly from the newborn nurseries. Thus, dependence on transport within the hospital and to the post office is eliminated. Furthermore, nursery personnel become accustomed to the schedule of the courier and make certain that the specimens are available for pickup. This routine has been in operation for several years and has proved to be more efficient and error-free then the aforementioned methods of transport.

Pitfalls in the Laboratory

PROCEDURAL ERRORS

In most screening laboratories, after the specimens have been logged in, filter paper discs are automatically punched into test tubes or microtiter plates from the circles of dried blood that impregnate the paper. Any misstep at this point can adversely affect the outcome of the assay and lead to mistakes in diagnosis. Even laboratories that manually punch specimens are not immune from the possibility of error. Some of the more egregious

maneuvers that have been associated with problematic laboratory results are: 1) a disc in the wrong test tube or microtiter well, 2) a disc in a tube or microtiter well that contains a previously punched disc, 3) a disc punched from an unsatisfactory specimen that contains insufficient blood, and 4) a tube or microtiter well that is processed while empty.

Each of these mistakes has the potential to generate false-negative or false-positive results, depending on whether the assay is for T4 or TSH. For example, the T4 or TSH results from a disc in the wrong test tube (or microtiter well) would be mismatched with a newborn, thereby leading to a potential error in diagnosis. Two discs in the same tube or well could result in a falsely elevated TSH result for a normal infant. Conversely, two discs in the same tube from an infant who has congenital hypothyroidism could result in a false-normal T4 value, missing the need for an assay for TSH. A disc from an infant who has hypothyroidism that contains insufficient blood could result in a normal instead of an elevated TSH value. On the other hand, a disc that contains insufficient blood from a normal infant would generate a low T4 value. Results from the processing of an empty tube or microtiter well depend on the assay methods and, therefore, are unpredictable. Avoiding these types of missteps requires a sense of commitment and freedom from distraction during repeated inspections of the tubes or plates before and after the specimens are punched.

Other potential shortcomings of automated assays are related to the degree of reliability of the equipment and the computers that control the operation. Because some of the steps of the procedure can be hidden from view of the operator, there is always the potential of an undetected instrument malfunction.

HUMAN ERROR

The advantage of using an automated assay for TSH or T4 is not just that it is less labor-intensive, but that there are fewer manipulations by individuals. Experience has shown that the greater the number of hands involved in a process, the greater is the likelihood of mistakes.

However, mistakes still can be made with automated assays. In either the automated or in-house assay for TSH or T4, test tubes or microtiter plates must be arranged and sorted by a technician. The exact location of the test tube in the rack or the precise sequence in the arrangement of the microtiter plates is the reference used to identify the specimens belonging to particular infants. If the test tubes or the racks are misaligned or the microtiter plates are out of sequence, there is a high probability of mismatching the specimen and the infant. Consider, for example, a situation in which an infant is found to have a low T4 level on the initial screen, which signals the need for a TSH determination. The specimen is punched and the discs added to a test tube or a microtiter well that is not in the proper sequence or position. Under these circumstances, if the infant who had the low T4 was hypothyroid, he or she would be found to have a normal TSH concentration. On the other hand, a specimen from a normal infant would have an elevated TSH level because the tube or well was identified with the incorrect infant.

Fortunately, most incidents such as these have been detected in time to prevent any diagnostic blunders. Experienced technicians examine the number of punch holes in the blood specimen from an infant who has an abnormal value. In the case described previously, the specimen from the normal infant had too few holes to have been used for both the T4 and TSH assays. The usual sequence after such discovery is to re-examine all specimens until the correct one is uncovered. To make absolutely certain that there are no other errors, the entire assay would be repeated.

In a slightly different but related process, mistakes can occur when assay results on the printouts are matched with the corresponding infants. A mismatch may occur during the transcription of values and their identifiers from the printout to the daily log. Information in the log indicates which specimens are to be retrieved for additional or repeat assays. If, for example, there is a low T4 value from a hypothyroid infant, but the matching identifier is incorrect, the wrong specimen will be retrieved for a TSH determination. Assuming the specimen is from a normal infant, the result of the TSH assay will be in the normal reference range. In this circumstance, the mistake usually is unnoticed, and the hypothyroid infant will be listed in the record as normal. Unfortunately, the diagnosis of congenital hypothyroidism is made months later when the clinical signs and symptoms of the disorder become apparent.

Attention to pitfalls in the laboratory underscores the fallibility of laboratory results. Clinicians never should rely on a single laboratory value to rule in or out the diagnosis of congenital hypothyroidism in a questionable case. In those instances in which clinical judgment conflicts with laboratory results, thyroid screening tests should be repeated.

Physician-related Pitfalls

Changes in medicine have caused the burden of responsibility for decision making in screening to be shared between the screening program and the physician. Many of the pitfalls that created diagnostic and therapeutic dilemmas for clinicians are largely the consequence of improved medical care. The introduction of pulmonary surfactant slightly more than one decade ago, plus advances in neonatal medicine, achieved striking declines in the mortality of low- and very low-birthweight infants. The dramatic increase in the rate of survival of even the tiniest of very low-birthweight infants (<1,500 g) has been a major reason for some of the more disturbing issues with which screening programs and physicians have had to struggle. To make informed decisions about results of newborn thyroid function tests, pediatricians must understand all factors that affect the outcome of those tests, including the advantages and disadvantages of initial tests used to screen for congenital hypothyroidism, the influence of birthweight on test results, delayed TSH elevations,

and the implications of transient hypothyroidism.

SCREENING TESTS

T4 as The Primary Marker

As mentioned earlier, most North American programs use a two-tier approach to screening in which T4 is the primary marker. Depending on the program, TSH concentrations are determined on those specimens whose T4 concentrations are in the lowest 5th to 15th percentile of the assays for a particular day. This approach lacks the specificity of the primary TSH strategy because so few of the low T4 values actually reflect thyroid insufficiency. However, a low T4 value can be an extremely useful marker because of its association with conditions such as TBG deficiency (approximately 1 in 4,000 births), prematurity and low birthweight, perinatal distress and systemic diseases, and pituitary and hypothalamic disorders (approximately 1 in 75,000 births).

Using the low T4 as a primary marker has the advantage of providing an additional clue to the early diagnosis of pituitary or hypothalamic disease. In a typical case involving a term infant, the initial screening specimen will have a low T4 and a low or normal TSH concentration. Faced with these values, it is incumbent on the physician to obtain additional blood specimens for repeat determinations of T4 and TSH. If the T4 remains low and the TSH is still normal, diagnostic possibilities include TBG deficiency or a hypothalamic-pituitary disorder. Failure to obtain the necessary blood specimens could prove detrimental to both infant and physician.

The lack of specificity of a low T4 concentration as a marker for thyroid insufficiency is a disadvantage for obvious reasons. Another less obvious but important disadvantage of the T4-TSH approach is that programs using this strategy cannot detect hypothyroidism in neonates who have abnormally high levels of TSH but normal T4 concentrations. An example of this is the hypothyroid infant who has an ectopic thyroid gland.

TSH as The Primary Marker

The specificity and sensitivity of TSH as a primary marker is indisputable, especially when screening term infants who are more than 2 days old. In addition, programs using this marker have the ability to detect infants who have elevated TSH levels and normal T4 concentrations, which is beyond the reach of most North American programs that use the two-tier approach.

Currently, a small number of North American programs use TSH as the primary marker to screen for congenital hypothyroidism. Such programs cannot identify infants who might have TBG deficiency, hypothalamic-pituitary disease, or some other condition in which the T4 is low.

Although TSH is undoubtedly the most specific and sensitive biochemical marker for the diagnosis of primary hypothyroidism, under certain circumstances its reliability suffers because of false-positive values. The physiologic surge of TSH after parturition often results in elevations that persist for as long as 48 hours. As a consequence, blood samples collected during this period may contain abnormally high concentrations of TSH, requiring the physician to recall the infant for repeat testing. Despite the increased probability of false TSH elevations, the clinician cannot afford to ignore such cases and possibly miss a truly hypothyroid infant.

Birthweight

Screening results in preterm and underweight infants cannot be considered in the same light as those from term infants. Studies in New England revealed that T4 concentrations in low-birthweight infants (1,500 to 2,500 g) and very low-birthweight infants (<1,500 g) varied directly with their weight. Surprisingly, 2% of the very low-birthweight group had T4 concentrations that were unmeasurably low, and 70% had T4 values in the hypothyroid range for term infants. These same studies revealed that the low T4 values in very low-birthweight infants resulted from decreased TBG concentrations. The fact that the incidence of permanent congenital hypothyroidism in the very low-birthweight group is no greater than that of the overall incidence is comforting. Although the free T4 level usually is normal in these infants, the question of whether hypothyroxinemia has an adverse effect on intellectual outcome is still debated.

The small number of programs that screen with primary TSH have not escaped the added burden created by the increased survival of preterm and underweight neonates. Very low-birthweight infants had eight times the incidence of abnormally elevated levels of TSH than did term infants in the New England study. However, as mentioned previously, these infants do not have a greater incidence of permanent hypothyroidism.

ATYPICAL HYPOTHYROIDISM

Until the early 1980s, the typical hypothyroid neonate identified at birth was characterized by an abnormally elevated concentration of TSH and a low level of T4 in the screening specimen. However, in 1983, six cases of congenital hypothyroidism were reported in which TSH profiles ran counter to this usual pattern. The TSH values were in the normal reference range at birth, but they increased to abnormally high levels several weeks later. Little was of particular note about the patients except that approximately 50% were preterm and underweight. Nothing more was heard about such "atypical cases" until a decade later, when the New England program described nine very low-birthweight infants whose TSH levels rose from normal at birth to abnormally high concentrations 2.5 to 7 weeks later. Since 1991, when the New England program first began tracking these cases, approximately 1 of 300 very low-birthweight neonates in Massachusetts has been found to have delayed elevations of TSH. These findings suggest that pediatricians should monitor all very low-birthweight infants closely in the perinatal period.

Speculation on the cause of this disorder includes the possibility of immaturity of the hypothalamic-pituitary axis or a transient condition induced by administered agents such

as iodine, dopamine, or large doses of corticosteroids. Regardless of the etiology, the need for active intervention should not be downplayed. The extent to which the central nervous system of the very low-birthweight infant is potentially vulnerable to damage during the interval between birth and the time of diagnosis and treatment is unknown. Because such infants are at increased risk of neurologic impairment, any action or inaction that might prove harmful should be avoided. To diagnose neonates who have atypical hypothyroidism in time to prevent potential central nervous system damage, the following guideline is recommended. In addition to initial screening tests, blood specimens should be obtained at 2, 6, and 10 weeks for repeat TSH and T4 determinations on all newborns weighing less than 1,500 g.

TRANSIENT HYPOTHYROIDISM

Transient hypothyroidism is defined as the maintenance of normal thyroid indices without treatment after demonstration of an abnormally elevated TSH. It should be noted that the incidence of this condition in Europe is relatively high, but it is low in North America. The causes are multifactorial and can be categorized as environmental, maternal, or neonatal in origin.

Both an excess and a deficiency of iodine are important environmental causes of transient hypothyroidism. Iodine overload through disinfection of maternal skin or use of iodized contrast media or topical iodinated antiseptics in infants, especially those of very low birthweight, can induce transient hypothyroidism. Although iodine excess can be a problem in North America, this is the only continent that does not have true iodine deficiency. Infants from iodine-deficient areas, when exposed postnatally to excess iodine, experience considerably more transient disease than do neonates in iodine-sufficient communities. This is consistent with the observation that iodine-deficient infants, especially those born preterm, are more sensitive to the suppressive effect of iodine on the thyroid.

One of the major maternal causes of transient disease in the newborn is the transplacental passage of antibodies from the mother that block the TSH receptor of both mother and infant. Other important causes in the neonate result from the maternal ingestion of antithyroid drugs or iodides, which readily cross the placenta and inhibit thyroid function. Infants whose mothers undergo treatment for Graves disease with antithyroid drugs also are at risk for transient hyperthyroidism from maternal thyroid-stimulating immunoglobulins. These infants may exhibit hypothyroidism on initial screening and hyperthyroidism on specimens obtained later for confirmatory blood tests. Generally it is not necessary to treat the transient disease induced by the previously cited drugs because thyroid function returns to normal within weeks.

Transient hypothyroidism that is unrelated to environmental or maternal causes, by process of elimination, is attributed to an intrinsic mechanism of the neonate. In most instances, the cause(s) never are defined.

In many cases, it is impossible to decide at the time of detection whether the hypothyroidism is permanent or transient. Even with clues that are associated with transience, such as TSH below 100 mU/L, male gender, pseudohyoparathyroidism, prematurity, iodine exposure, or dopamine administration, the diagnosis may be in doubt. Under such circumstances, it is best to manage the patient as if the hypothyroidism is permanent. If the diagnosis has not become apparent by the age of 3 years, T4 administration should be discontinued and the infant monitored with serial determinations of T4 and TSH.

MISCELLANEOUS PROBLEMS

Defects or lesions of the pituitary or hypothalamus can result in permanent secondary or tertiary hypothyroidism, respectively. Idiopathic hypopituitarism, isolated TSH deficiency, or any of the congenital syndromes associated with pituitary or hypothalamic dysfunction such as septo-optic dysplasia are some of the common etiologies of these rarer forms of hypothyroidism. The diagnosis is suggested by either clinical signs or a low free T4 concentration in the presence of a low, normal, or even slightly elevated TSH.

TBG deficiency is predominantly an X-linked disorder that occurs in approximately 1 in 4,000 births, with a male-to-female ratio of about 9:1. Laboratory clues to the diagnosis are a low total T4 but a normal free T4, an elevated resin T3 uptake, or a low TBG concentration. The parents should be reassured that the condition is innocuous and that at no time in the immediate future is treatment necessary.

Exchange transfusions have the capacity to lower an abnormally elevated TSH level, thereby obscuring the diagnosis of primary hypothyroidism. To avoid this pitfall, the specimen should be collected before or 48 to 72 hours after the transfusion. Another somewhat related problem is a twin-to-twin transfusion that can normalize an otherwise hypothyroid laboratory profile.

One of the least publicized but most important of potential pitfalls is failure to respond to requests for repeat blood samples in a timely fashion or at all. Too often the initial screening specimen is unsatisfactory for testing. Any delay in obtaining a repeat blood specimen prolongs the time between testing and making the diagnosis of hypothyroidism. A delay in diagnosis subsequently delays the initiation of treatment. This is antithetical to the goal of all screening programs, which is to begin treatment as close to birth as possible.

Conclusion and a Final Admonition

Potential pitfalls in screening for congenital hypothyroidism are ubiquitous, ranging in scope from the birth hospital to the screening laboratory to the pediatrician's office. Recognizing where and how mistakes can be made is the best means of preventing them.

A final word regarding the potential pitfall that is of the physician's own making when managing a newly diagnosed infant who has congenital hypothyroidism. During the first year of life, it is crucial that the circulating level of T4 remain in the upper middle half of the refer-

ence range of normal. Failure to obtain frequent measurements of T4 concentrations could result in protracted periods of abnormally low circulating levels of T4 that conceivably could compromise developmental outcome (see American Academy of Pediatrics guidelines in Suggested Reading).

Acknowledgment

I thank Ms. Rosemarie Collard for her skill and patience in the preparation of this manuscript.

SUGGESTED READING

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NEOREVIEWS QUIZ

Quiz also available online at www.neoreviews.org.

3. Although screening programs for congenital hypothyroidism are well established, there are potential pitfalls in implementation of findings. Of the following, the *most* accurate statement regarding newborn screening for congenital hypothyroidism is that:
   - A. Assays using laboratory personnel are prone to fewer errors than automated assays.
   - B. Blood samples should be collected within 24 hours of birth to obtain reliable results.
   - C. Hospital couriers are more reliable than commercial couriers for transfer of blood specimens.
   - D. Insufficient blood volume on filter paper disks can yield false-positive and false-negative results.
   - E. Screening is less likely to be missed in neonates who are transferred to special care facilities.

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