Sani-Cloth Wipe Mimics Rare Enzyme Deficiency Malonic Aciduria on Newborn Screen

abstract

Within a 7-month period at our institution, newborn screening by tandem mass spectrometry revealed 10 cases with elevated levels of malonylcarnitine, which suggested malonic aciduria. Malonic aciduria is a rare autosomal recessive inborn error of metabolism. Confirmatory testing yielded normal results in all the newborns involved. The application of quality improvement practices dictated investigating the dried blood spot collection process, which revealed the use of multiple blood-collection techniques by newborn nursery staff, improper handling of the dried blood spot specimens, and sanitary wipe contamination as the causes of the aberrant false-positive results at our institution. This systematic evaluation identified the cause of the aberrant false-positive results and a strategy was implemented to avoid aberrant results in the future. Thus far, no false-positive results have occurred since the investigative process. False-positive results on a newborn screen can cause unnecessary emotional and economic stress on families, a finding that was identified at our institution. Historically, false-positive newborn screening results have been identified in infants born by cesarean delivery in which iodine antiseptic was used and in newborns who receive total parenteral nutrition, such as premature infants in the NICU. Therefore, if an unusually high number of false-positive results are found during the newborn screening process, contamination should be considered as a contributing factor. Pediatrics 2012;130:e1363–e1368
Newborn screening is recognized as an essential, preventive public health program for the early identification of genetic and metabolic disorders in newborns. According to the National Newborn Screening and Genetics Resource Center, the early detection, diagnosis, and treatment of certain genetic, metabolic, or infectious congenital disorders can lead to significant reductions in death, disease, and associated disabilities. States routinely test dried blood spots collected from newborns for at least 30 metabolic and genetic conditions, as recommended by the American College of Medical Genetics and the US Department of Health and Human Services.

Malonic aciduria (MAL), also known as malonyl-CoA dehydrogenase (MCD) deficiency, is a rare autosomal recessive inborn error of metabolism with fewer than 30 cases described in the literature before the widespread use of tandem mass spectrometry (MS/MS) analysis. No known population is at an increased risk of inheriting the disease and the age of presentation ranges from a few days to 13 years of age. Before newborn screening for MAL, only symptomatic patients had been detected and usually presented with significant developmental delay. Acute cardiac decompensation (hypertrophic and/or dilated cardiomyopathy), hypotonia, hypoglycemia, metabolic acidosis, diarrhea, vomiting, and seizures can also be presenting factors. Accumulation of malonic acid, as well as a secondary carnitine deficiency, which are the result of a deficiency of MCD, are responsible for the signs and symptoms of the disease. Because of the expansion of newborn screening and the use of MS/MS analysis, additional cases of MAL are identified without the usual disease symptomatology owing to the heterogeneity of the disease.

Elevated plasma malonylcarnitine by MS/MS analysis and elevated urine malonic acid by gas chromatography–mass spectrometry analysis are considered diagnostic for MCD deficiency. Confirmatory tests, such as the measurement of MCD activity in cell extracts or molecular genetic testing, may then be used. Mutations within the MLYCD gene on chromosome 16q24 have been linked to MCD deficiency but no common mutations have been identified at this time.

**PATIENT PRESENTATIONS**

Within a period of 7 months (in 2009), 10 potential cases of MAL with increased levels of malonylcarnitine (C3-DC) on newborn screen were reported to our institution based on MS/MS analysis conducted at the State Hygienic Laboratory at the University of Iowa (SHL) Newborn Screening (NBS) Laboratory (Table 1). In contrast, during the previous year, only 2 newborn screens exceeded the cutoff value for C3-DC by MS/MS. No cases were found in the other 2 state programs tested by the SHL NBS Laboratory (Iowa and North Dakota) during this time. Confirmatory testing demonstrated no evidence of disease in any of the 10 cases.

The SHL NBS Laboratory conducted a Define, Measure, Analyze, Improve, and Control approach to investigate the false-positive results. The problem, at that time, was defined as too many false-positive results from a single institution. The NBS laboratory measured the problem by requesting data on special feeding and/or collection practices, to determine probable causes of the elevated C3-DC levels. Analyzing the false-positive newborn screens revealed that no special feeding of the newborns could provide interference, but that multiple nurses had collected the newborn blood specimens onto the standard filter paper collection forms using varying blood draw techniques. To help improve the collection process, the SHL NBS Laboratory staff stressed to hospital laboratory supervisors that Standard Work Practices need to be followed by all collection staff according to the Clinical and Laboratory Standards Institute–published guidelines, ensuring that proper blood collection and drying techniques were used. Supervisors reviewed dried blood spot collection technique with the nursery staff to ensure that the collection process was uniform among nurses to help diminish the possibility of a pre-analytical error as the cause of the aberrant false-positive results.

During the review of blood draw technique by the newborn nursery staff, it was noted that the filter paper used for blood collection was placed on a countertop that had recently been sanitized using a Sani-Cloth wipe (PDI Inc., Orangeburg, NY). It was at this time that SHL NBS Laboratory personnel were informed that the nursery had recently switched to Sani-Cloth wipes from a different disinfectant. It was hypothesized that the fresh blood from the filter paper was contaminated with

<table>
<thead>
<tr>
<th>Patient</th>
<th>C3-DC Screen Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (µM in Blood)</td>
<td>≥0.20 µM = Presumptive Positive</td>
</tr>
<tr>
<td>1</td>
<td>0.22</td>
</tr>
<tr>
<td>2</td>
<td>0.21</td>
</tr>
<tr>
<td>3</td>
<td>0.26</td>
</tr>
<tr>
<td>4</td>
<td>0.25</td>
</tr>
<tr>
<td>5</td>
<td>0.20</td>
</tr>
<tr>
<td>6</td>
<td>0.20</td>
</tr>
<tr>
<td>7</td>
<td>0.31</td>
</tr>
<tr>
<td>8</td>
<td>0.28</td>
</tr>
<tr>
<td>9</td>
<td>0.23</td>
</tr>
<tr>
<td>10</td>
<td>0.21</td>
</tr>
<tr>
<td>11 (Newborn with normal acylcarnitine profile)</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Samples of Sani-Cloth wipes were sent to the SHL NBS Laboratory for MS/MS analysis. Liquid from the wipes was collected and dilutions of 1:20, 1:50, 1:100, and 1:200 were prepared with deionized high-performance liquid chromatography-grade water. The diluted disinfectant was spotted on filter paper collection cards and dried. The dried disinfectant dilutions were sampled, extracted, and analyzed by electrospray MS/MS using stable isotope internal standard methodology in the same manner as the newborn dried blood specimens (Hardy DT. Technological Developments of Tandem MS for Neonatal Screening. West Midlands Regional Laboratory for Neonatal Screening and Inherited Metabolic Disorders, Department of Clinical Chemistry, Paediatric Laboratory Medicine Block, Birmingham Children's Hospital NHS Trust, unpublished internal technical report, July 1999), with butylated derivatives of the acylcarnitines detected and quantitated by multiple reaction monitoring (Figs 1, 2, 3, and 4). Dilutions up to 1:50 of the Sani-Cloth disinfectant produced a C3-DC result above the newborn cutoff of 0.20 μM (Table 2). Therefore, it was possible that the falsely elevated C3-DC level was caused by only a few microliters of undiluted disinfectant within the dried blood specimen.

The active ingredients used in the Sani-Cloth product are quaternary ammonium compounds, which chemically consist of nitrogen cations covalently bonded to alkyl groups,
some of which contain long carbon chains. Disinfectants with quaternary ammonium compounds as the active ingredient are commonly used in hospitals for surface decontamination. We have not determined the mechanism by which the chemicals derived from the disinfectant interfere in the MS/MS methodology, but precursor and fragment ions of similar mass/charge ratio contributed to the peak signal for the C3-DC acylcarnitine.

After identification of the Sani-Cloth contamination of newborn blood specimens, the proper technique for filter paper blood collection was once again reviewed with nursing staff. It was emphasized that the filter paper should never contact or touch a surface during the collection or drying process and the protective flap must be used to hold the filter paper above the countertop. It was also discussed that the filter paper should never be handled with bare or gloved hands and the nursery staff needed to change gloves after sanitizing the countertop with Sani-Cloth wipes. This information was in turn communicated to the NICU nursing staff to make them aware of the potential for false-positive results owing to chemical contamination. No false-positive C3-DC results have occurred at our institution after the investigative process.
DISCUSSION

Newborn screening is an important public health measure that has expanded in South Dakota from 3 disorders in 1995 to 44 disorders in 2005. The expansion of newborn screening allows for increased detection of genetic disorders, but an increase in false-positive results is a subsequent consequence. It is estimated that the false-positive rate in newborn screens that test for >20 disorders is 0.33% with an overall incidence of ~1 case per 2400 infants. In 2005, it was estimated that the number of false-positive results in South Dakota that year could range from 12 to 247 false-positive results. The described 10 false-positive results occurred over a 7-month period; however, the 10 false-positive results occurred on a single test and do not incorporate the other disorders on the newborn screen. In addition, South Dakota had only 2 false-positive results for C3-DC the previous year. Because of these factors and the fact that MAL is an extremely rare disorder, investigation into the newborn screening process was correctly initiated.

Fortunately, all of the cases were false-positive results; however, false-positive results on a newborn screen can lead to increased parental stress. One study demonstrated that mothers of children with a false-positive screen worried more about their child’s future, rated themselves as less healthy, and felt that their child required extra care. In addition, both parents had higher overall stress on the parenting stress index. At our institution, one family related their experience as being emotionally traumatic, and financial stress on the families was evident as confirmatory studies totaled $693.40 per infant. Evaluation of these false-positive results revealed that contamination by a substance in sanitary wipes was responsible for the results. Previous studies have also documented contamination as a cause of false-positive results on newborn screening. Iodine antiseptic used for cesarean deliveries has been shown to result in a higher average value of neonatal thyrotropin and a higher percentage of neonatal specimens with a thyrotropin greater than or equal to 5 mIU/L. Contamination of blood with total parenteral nutrition can potentially result in an elevation of false-positive results because of the presence of amino acids. Furthermore, higher rates of false-positive results owing to elevated amino acids or short-chain acylcarnitines have been observed in laboratories whose screening population includes premature infants on total parenteral nutrition. However, no documented cases in the literature have identified sanitary wipes as a potential cause of false-positive newborn screening results.

To reduce the number of false-positive results on a newborn screen and the significant emotional and economic stress for families, the follow-up team of doctors and nurses need to work closely with the laboratory and use defined quality measures to guarantee success. The Define, Measure, Analyze, Improve, and Control approach is the quality measure that defined, measured, and analyzed the problem of an unusual number of false-positives. This approach emphasized using Standard Work Practices to improve the collection and handling of NBS specimens, which will control the process and exclude contamination in the future.

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