Newborn Screening for Isovaleric Acidemia Using Tandem Mass Spectrometry: Data from 1.6 Million Newborns

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BACKGROUND: Electrospray ionization–tandem mass spectrometry (ESI-MS/MS) has been used in the Bavarian newborn screening (NBS) program since 1999. The use of ESI-MS/MS has led to the inclusion of isovaleric acidemia (IVA) into NBS. We retrospectively evaluated data on more than 1.6 million newborns screened during 9.5 years.

METHODS: Acylcarnitines from whole blood spotted on filter paper were converted to their corresponding butyl esters, and the samples were analyzed by use of ESI-MS/MS with stable isotope labeled internal standards.

RESULTS: A total of 24 individuals with IVA were detected by use of a multiparametric threshold criteria panel including isovalerylcarnitine (C5) and the ratios of C5 to octanoyl-, butyryl-, and propionylcarnitine. A cutoff set at the 99.99th percentile for isolated C5 or at the 99th percentile for C5 plus at least 2 ratios resulted in a positive predictive value for IVA screening of 7.0% and an overall recall rate of 0.024%. Adjusted reference ranges for age and birth weight were applied, and the incidence of IVA in the study population was calculated to be 1 in 67,000. Missed cases were not brought to our attention. IVA was also detectable in cord blood and early postnatal blood samples.

CONCLUSIONS: IVA can be reliably detected in NBS through acylcarnitine analysis in dried blood spots by using multiparametric threshold criteria. Further improvement (positive predictive value 13.0%, recall rate 0.01%) can be achieved by using more stringent recall criteria. In view of the potentially life-threatening natural course of IVA in early life, presymptomatic diagnosis may thus prevent mortality and morbidity.

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Isovaleric acidemia (IVA) 4 (OMIM #243500) is caused by a defect in leucine catabolism due to deficiency of isovaleryl–coenzyme A (CoA) dehydrogenase, leading to the accumulation of free isovaleric acid, 3-hydroxyisovaleric acid, N-isovalerylglycine, and isovalerylcarnitine (C5). Characteristic but unspecific clinical signs include episodes of recurrent vomiting, and there is a risk of coma and death. IVA manifestation varies from the acute neonatal type, with early onset of metabolic decompensation, to the chronic intermittent type, with onset in infancy or childhood that presents as developmental delay and/or failure to thrive (1).

IVA has been part of the newborn screening (NBS) disease panel in Bavaria since 1999, with C5 used as the marker metabolite. However, isobaric acylcarnitines like 2-methylbutyrylcarnitine and pivaloylcarnitine (2,2-dimethylpropionylcarnitine) cannot be distinguished by the screening method. Therefore, NBS for IVA may also detect patients with 2-methylbutyryl-CoA dehydrogenase (short/branched-chain acyl-CoA dehydrogenase) deficiency (SBCADD, OMIM #610006) caused by mutations in acyl-CoA dehydrogenase, short/branched chain (ACADSB) (600301) (2). This metabolic condition is of uncertain clinical relevance and is not part of the German NBS disease panel, the use of which became required by law in 2005. In addition, the administration of antibiotics containing

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Nonstandard abbreviations: IVA, isovaleric acidemia; CoA, coenzyme A; C5, isovalerylcarnitine; NBS, newborn screening; SBCADD, short/branched-chain acyl-CoA dehydrogenase deficiency; PPV, positive predictive value; ESI-MS/MS, electrospray ionization–tandem mass spectrometry; C8, octanoylcarnitine; C4, butyrylcarnitine; C3, propionylcarnitine.
pivalic acid derivatives may lead to false-positive screening results (3).

Published data on the incidence of IVA, recall rate, and the positive predictive value (PPV) of NBS for IVA are heterogeneous. In particular, the procedure of establishing cutoff values is often not explained in detail (4–9), or it is arbitrarily set to a certain value (10). This study provides comprehensive data on the performance of electrospray ionization–tandem mass spectrometry (ESI-MS/MS)-based NBS for IVA from 1 612 105 newborns analyzed at the NBS laboratory in Munich, Germany, during a 9.5-year period.

Free carnitine and acylcarnitines were quantified as described previously (11) with minor modifications (see the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol57/issue4). For the interpretation of acylcarnitine profiles in NBS for IVA, C5, and the ratios of C5/octanoylcarnitine (C8), C5/butyrylcarnitine (C4), and C5/propionylcarnitine (C3) were used. Data on the linearity, imprecision, recovery, analytical sensitivity, and detection limit of the assay are provided in the online Data Supplement. Individual cutoffs were set to the 99th percentile (online Supplemental Table 1). Isolated increases of the blood C5 concentration above the 99.99th percentile (>1.37 μmol/L) or the increase of the blood C5 concentration between the 99th (0.51 μmol/L) and the 99.99th percentile in addition to increased values of at least 2 relevant acylcarnitine ratios resulted in a recall, whereas increases of only 1 or 2 of the 4 values did not.

A total of 24 cases of IVA and 3 cases of SBCADD were detected (Fig. 1). A fourth case of SBCADD was identified during family investigations; the C5 concentration in the NBS sample from this patient had been below the cutoff. Among the 24 cases of IVA, 11 were defined as “metabolically mild or intermediate” (NBS C5 concentration: median 4.0 μmol/L, range 0.8–4.8 μmol/L) and 11 as “metabolically severe” (NBS C5 concentration: median 14.4 μmol/L, range 8.0–22.1 μmol/L; 2 patients with a positive family history were excluded because of carnitine treatment initiated before NBS blood sampling), according to a classification suggested previously (11). This categorization was recommended to be used as a basis for treatment decisions. In all patients with the metabolically mild or intermediate type of IVA who had mutation analysis (64%, 7 of 11) the common c.932C>T (p.A282V) mutation (12) was identified in either a homozygous or compound heterozygous state (allele frequency 64%; 9 of 14). In contrast, none of the patients with metabolically severe IVA who underwent genotyping (91%, 10 of 11) carried the c.932C>T mutation.

The resulting total incidence for IVA was 1:67 000 (95% CI: 1:45 000–1:107 000), whereas the total incidence for SBCADD was 1:403 000 (95% CI: 1:161 000–1:1 612 000). The overall recall rate for C5 and related metabolite ratios was 0.024%. The diagnostic specificity was 99.98% for both IVA and SBCAD screening. The diagnostic sensitivities were 100% and 75% for IVA and SBCADD, respectively. The PPV for IVA screening was 7.0%. At the time of this report, no missed cases of IVA had been reported. In addition, detection of IVA in cord blood and early postnatal blood samples was possible (see the online Data Supplement). Although the clinical utility of cord blood testing for several inherited metabolic disorders was investigated in one recent study (13), corresponding data for the diagnosis of IVA were not available, because no patients with IVA were enrolled in this study.

The investigation of a population of more than 1.6 million neonates provided the basis for evaluating the efficiency of the Bavarian NBS program for IVA. The data set enabled us to calculate the IVA incidence and to provide age- and birth-weight–dependent reference values for the marker metabolite C5 and the related metabolite ratios C5/C8, C5/C4, and C5/C3 (online Supplemental Figs. 1 and 2, online Supplemental Tables 1 and 2, and Results in the online Data Supplement). Data obtained during the first 6 years of this study led to the inclusion of IVA into the standard NBS panel in Germany in 2005 (14).
Earlier NBS reports on the incidence of IVA included data sets with information from sample sizes between 160,000 and 1,500,000 newborns (Table 1). However, the number of confirmed cases of IVA per study was rather small (0–7) compared to the 24 cases of IVA identified among 1.6 million newborns in our study. Thus, the reported incidences of IVA in the different programs vary significantly between 1:62,500 and 1:362,000, with 95% CIs ranging from 1:24,000 to 1:72,000. Incidences from our study (1:670,000, 95% CI 1:65,000–1:335,000) are comparable to those previously reported from Heidelberg (1998–2001) (1998–2001) and those compiled for Germany as a whole (2004–2006) by the German Society for Newborn Screening (2004–2006). Our data may thus also reflect the situation in Germany, which we attempted to depict by summarizing results from all 3 available data sets (Table 1). Comparable incidences were identified in a large cohort screened in North Carolina (6). In general, the incidence tends to be lower in smaller sample populations, and incidence differences observed in different regions of the world may in future vanish as increasing numbers of newborns are screened.

Further comparison of IVA NBS results arising from different programs is challenging due to the often incompletely reported data regarding sensitivity, PPV, and recall rate. Within the 9.5 years of the follow-up period of our NBS program, no cases of false-negative IVA-screening results have come to our attention, suggesting a high diagnostic sensitivity of the procedure. Although sample sizes differed greatly, recall rates reported from New England, Pittsburgh, North Carolina, Heidelberg, and the German Society for Newborn Screening were all in the range between 0.01% and 0.05%, similar to our study (0.024%) (Table 1). However, only limited details are available on the procedures that were used to set up cutoff values. The PPV was provided for 3 of 8 studies, and ranged from 1.8% to 10.8% (our study, 7.0%). This variation was due to a relatively high number of false-positive IVA screening results, as seen in other NBS programs (6); Table 1). Although the majority of NBS programs applied C5 as the primary biochemical marker in the screening for IVA, only one other program reported the use of multiparametric threshold recall criteria including C5 and secondary related ratios C5/C8, C5/C4, and C5/C3 (8), such as those used in the Bavarian NBS program. We calculated various additional ratios (such as C5 to acetylcarnitine, free carnitine, or various long-chain acylcarnitines), which did not help to further increase the discriminatory power. A change in the recall policy could improve the recall rate and the PPV. With the application of only values of C5 above the 99.99th percentile (>1.37 μmol/L), or C5 and the 3 ratios (C5/C8, C5/C4, and C5/C3) instead of 2 ratios, all above the 99th percentile, the recall rate for IVA could be lowered from currently 0.024% to 0.01%, increasing the PPV from 7.0% to 13.0%. With this strategy all cases of IVA,

<table>
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<th>Data from</th>
<th>Nb</th>
<th>Recall rate, %</th>
<th>FP</th>
<th>TP</th>
<th>PPV, %</th>
<th>Incidence</th>
<th>95% CI of incidence</th>
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<td>&lt;6</td>
<td>—</td>
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<td>—</td>
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<td>1:72,000</td>
<td>1:49,000–1:109,000</td>
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<td>Munich, Germany (present study)</td>
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<td>7.0</td>
<td>1:67,000</td>
<td>1:45,000–1:107,000</td>
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**Summary**

1. Heidelberg
2. Germany 2004–2006
3. Munich (present study)

*a Values in bold are taken from the respective literature and used to calculate or estimate additional data listed.
*b N, number of newborns tested; FP, number of false positives; TP, number of true positives (IVA confirmed); PPV, positive predictive value.
1 Annual national screening reports of the German Society for Newborn Screening (DGNS), 2004–2006. Data from our study are partly included.

Data from all 3 German studies were pooled to calculate the incidence and the 95% CI of the incidence. Duplicate data from Bavaria were removed.
inclusion of the variant cases, but only 1 of the 4 cases of SBCADD would have been detected.

Such a strategic change is in line with the current German NBS disease panel initiated in 2005, which does not include SBCADD. This decision rested upon the questionable benefit of neonatal screening for SBCADD, considering that the exact natural history and pathogenicity of this condition have not been delineated to date. The suggestion that SBCADD is a benign biochemical variant has been based on the finding that individuals identified by NBS have remained asymptomatic so far, even without treatment, and also on the identification by family screening of asymptomatic individuals with the condition (2, 17). Although it cannot be entirely excluded that symptoms could possibly be triggered by metabolic stress, as is known to occur with other metabolic disorders, the diverse clinical problems reported in affected individuals may in fact be coincidental. Furthermore, it is likely that in most NBS programs cases of SBCADD may not be detected, given the discrepancy between the relatively high cutoff levels reported for C5, ranging from 1.16 to 2.0 μmol/L (5, 6, 9, 10), and the only mildly increased C5 concentrations identified in patients with SBCADD (our study, 0.26–0.91 μmol/L).

In conclusion, we used a large cohort to assess the efficiency of NBS for IVA through the analysis of a multiparametric acylcarnitine panel in dried-blood spots. The PPV was 7.0% and the recall rate 0.024%, which can be improved by the application of more stringent recall criteria to a PPV of 13.0% and a recall rate of 0.011%. Considering that IVA can be associated with substantial morbidity and mortality at first presentation, potentially occurring at the end of the first or in the second week of life, it is an ideal disorder to be diagnosed presymptomatically by prospective NBS.

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