

NEW TRENDS IN NEWBORN SCREENING THE EXAMPLE OF LYSOSOMAL STORAGE DISORDERS

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Newborn screening has been implemented worldwide for many different inherited disorders, including inborn errors of metabolism, endocrinopathies, hemoglobinopathies, cystic fibrosis and others. While expanded newborn screening by tandem mass spectrometry for analysis of acylcarnitine species, and amino acids is widely available, additional novel applications are currently being developed and tested. With the advent of novel treatment modalities in lysosomal storage diseases (LSD) such as bone marrow transplantation and/or enzyme replacement therapies, newborn screening for some of the LSD for example Pompe Disease, Fabry Disease, Gaucher Disease, Krabbe Disease and others has become a focus point. From a technological perspective high - throughput newborn screening for LSD may be feasible using different analytical approaches, such as the analysis of enzyme activities by fluorometric assays, tandem mass spectrometry or analysis of protein (enzyme) concentrations by protein binding assays, respectively. Routine newborn screening for Fabry and Pompe Diseases is currently been done in parts of Taiwan and for Krabbe Disease in New York State. Additional programmes are in the process of being implemented. However prior to wide spread implementation of neonatal screening programmes for LSD, strategies for confirmatory testing, treatment, follow-up care and scientific evaluation have to be defined and agreed upon at an international level.

Descriptors: NEWBORN SCREENING, NEW TRENDS, LYSOSOMAL STORAGE DISORDERS

Introduction

Lysosomal storage disorders (LSD) comprise a heterogeneous group of more than 50 different disorders that are due to a deficient enzyme, transporter or membrane protein (1). They are characterized through progressive intra-lysosomal storage of metabolites in different organ systems resulting in a variety of clinical symptoms, increased morbidity and mortality (1). The majority of LSD shows significant central nervous involvement (1). Enzyme replacement therapies are currently available for 6 different LSD including MPS-I, II, VI, Fabry,

Gaucher and Pompe Disease respectively and are being developed for others. In addition bone marrow and/or chord blood transplantation may be indicated for the treatment of MPS-I and Krabbe disease respectively (1). There is some anecdotal evidence that early diagnosis and treatment may have the potential to alter the natural history and reduce morbidity and mortality. In this context it may be useful to implement newborn screening for LSD although many issues, such as phenotype prediction, follow-up and the diagnosis of late-onset cases remain unsolved at present.

Three different analytical methods; tandem mass spectrometry, immune capture and flurometry have been propagated for diagnosis of LSD in dry blood spots (2-6).

The use of tandem mass spectrometry (MS/MS) for enzyme analysis in dry blood spots for the diagnosis of Fabry, Gaucher, Krabbe, Niemann-Pick and Pompe disease respectively has been

recently evaluated (2-4). The following review will focus on the newborn screening for LSD using either tandem mass spectrometry or flurometric methods to measure enzyme activities. The analytical protocols can be found in more detail elsewhere (4, 6). In brief, analyses by tandem mass spectrometry can be multiplexed for different LSD, but is time consuming and requires expensive equipment (4). Flurometric analysis on the other hand is relatively easy to perform, requires less expensive equipment but may not be multiplexed and is therefore not suitable for high throughput analysis of a larger number of LSD (6).

Pompe Disease (PD)

A PD newborn screening pilot program in Taiwan used acarbose and 4-MUG to measure glucosidase alpha (GAA), the enzyme deficient in PD, activity in dry blood spots (DBS) (6). The screening program covers about 45% of the Taiwanese population while the

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same laboratory provides Pompe diagnostic services for all of Taiwan. Between October 2005 and March 2007 more than 130,000 newborn infants were screened and PD was diagnosed in 4 infants during their first month of life. In contrast 3 infants were diagnosed during the same time period based on clinical symptoms alone between 3 and 6 months of age. All infants except one in the screening group had infantile-onset PD and were started on enzyme replacement therapy (6). The recall rate for repeat blood tests was 0.82% and for clinical recall 0.091% (6, 7). Outcome in the newborn screening group is excellent with all children achieving normal to near-normal psychomotor development. These data will be published soon (Paul Hwu personal communication).

The MS/MS technique for GAA analysis in DBS was further evaluated and validated on more than 10,000 anonymous newborn infants in Austria and 29 known patients with PD (2). The recall rate in this study was 0.03%, which compares very low to the results of newborn screening by fluimetry (2, 6). The MS-MS technique for newborn screening of PD needs to be evaluated in a larger, preferably nationwide pilot study.

Krabbe Disease (KD)

New York State Laboratories, Wadsworth Center Albany, NY has started newborn screening for KD using MS/MS technology in August 2006. Through June 2008 the Wadsworth Center has tested more than 550,000 newborn infants and identified four infants at high risk for developing early onset KD (4, 8, 9). Two of these infants with severe pathogenic mutations in the *galactocerebrosidase* gene and neuroradiologic findings received umbilical chord transplantation within the first 4 weeks of life. Unfortunately one infant died during transplantation due to procedure related complications (8). The other infant has not developed any signs or symptoms related to early onset KD but are developmentally delayed (8). The 2 high-risk infants who were not transplanted, show age-appropriate psycho-motor development at 8 and 16 months of life, respectively (8).

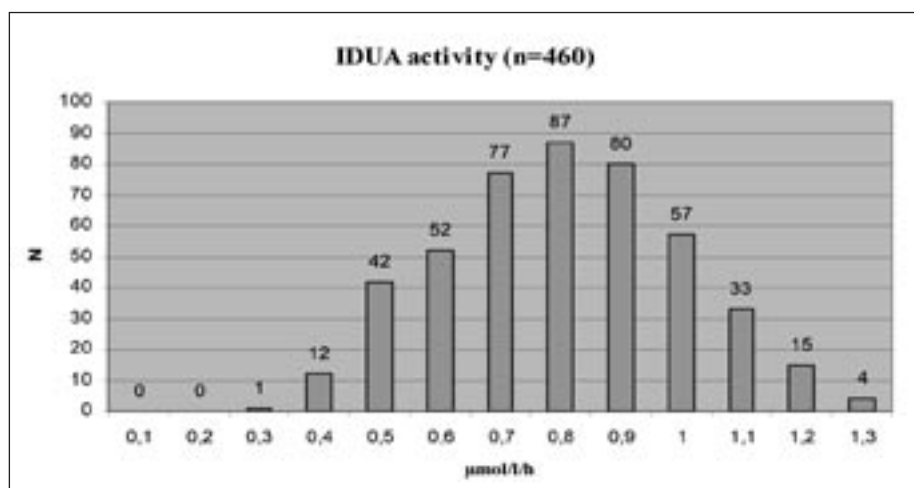


Figure 1
α-Iduronidase activity in 460 newborn infants; one infant with a severe form of MPS-I (Hurler Phenotype) had no detectable enzyme activity

Slika 1.

α-Iduronidazna aktivnost kod 460 novorođenčadi; jedno novorođenče sa teškim oblikom MPS-I (Hurler Phenotype) nije imalo prepoznatljivu enzimnu aktivnost

Mucopolysaccharidosis Type I (MPS-I)

The microplate adapted fluimetric, iduronidase assay is easy to perform and seems to be suitable for large scale pilot newborn screening (10). We have done a small pilot study using this protocol on 460 anonymous infants and demonstrated a median iduronidase activity of 0.8 μmol/l/h while the single MPS-I patient tested, had no measurable enzyme activity (Figures 1 and 2). Based on our limited experience recall rates appear low so that this protocol may be suitable for large scale newborn screening.

Fabry Disease (FD)

Two pilot studies using a fuimetric assay for FD were done or are still ongoing. A pilot study in northern Italy identified 12 neonates with FD among 37,000 male infants. The vast majority of infants (11/12) with FD had mutations compatible with a mild disease course. In addition affected family members were detected. A similar pilot study for Fabry disease in Taiwan is ongoing, details are currently not available.

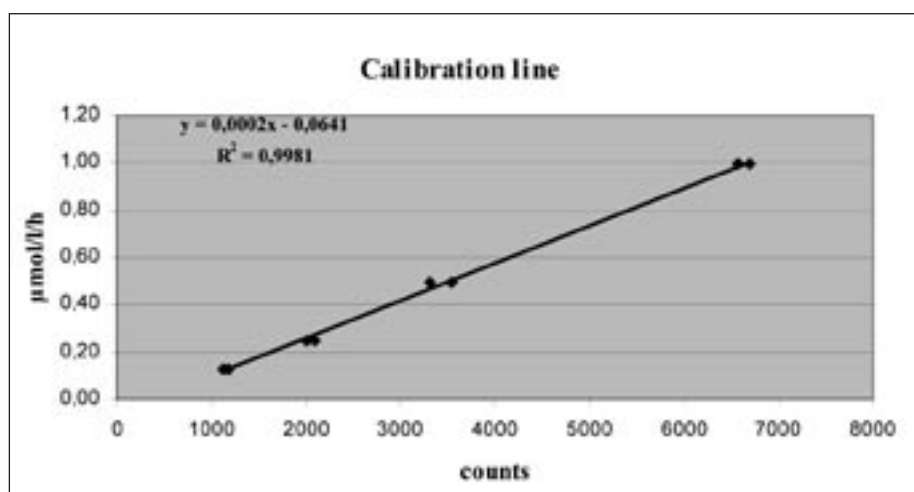


Figure 2
Calibration line of the α-Iduronidase fluorimetric assay

Slika 2.

Linija kalibracije α-Iduronidazne fluorometrijske analize

Conclusions

Although newborn screening for LSD using different analytical approaches may be technically feasible, it is still a long way to go. Careful planned pilot studies in large newborn populations are needed to assess test characteristics, define population specific cut-off values and diagnostic algorithms. Prior to formal implementation of newborn screening for LSD recommendations and guidelines for confirmatory testing, management and long-term follow up have to be agreed upon, preferably at an international level. The Krabbe Disease newborn screening and follow-up programme in New York State may serve as a model (8).

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Sažetak

NOVI TRENDovi U SKRININGU NOVOROĐENČADI PRIMJER POREMEĆAJA LIZOSOMNIH BOLESTI

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Skrining novorođenčadi se primjenjuje u čitavom svijetu za mnoge različite nasljedne poremećaje, uključujući urođene greške u metabolizmu, endokrinopatije, hemoglobinopatije, cističnu fibrozu i drugo. Dok je prošireni skrining novorođenčadi putem tandemске spektrometrije masa za analizu acilokarnitinskih vrsta i aminokiselina širom dostupan, trenutno se razvijaju i testiraju neke nove aplikacije. Dolaskom novih modaliteta liječenja lizosomnih bolesti (LSD), kao što su transplantacija koštane srži i/ili terapije zamjene enzima, skrining kod novorođenčadi za neke LSD kao što su Pompe bolest, Fabry bolest, Gaucher, Krabbe i dr. su postali interesantni. Tehnološki gledano, visoko propusni skrining novorođenčadi na LSD može biti izvediv koristeći se raznim analitičkim pristupima, kao što su analiza enzimskih aktivnosti putem fluorometrijske analize, tandemске spektrometrije masa ili analiza proteinske (enzimske) koncentracije putem analize vezivanja proteina. Rutinski skrining novorođenčadi za Fabryevu i Pompeovu bolest se trenutno obavlja u nekim dijelovima Taiwana a za Krabbeovu bolest u državi New York. Dodatni programi su u procesu uvođenja. Međutim, prije široke primjene programa neonatalnih skrininga na LSD, moraju se definirati strategije i odrediti međunarodni konsenzus za testiranje, liječenje, oporavak i znanstvenu evaluaciju.

Deskriptori: NOVOROĐENAČKI SKRINING, NOVI TRENDovi, LIZOSOMNE BOLESTI