

Symposium ‘Rare metabolic diseases’

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National Programme for Rare Diseases 2015- 2016

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Rare diseases (RD) are a heterogeneous group of conditions defined by their prevalence. In the European Union, a disease is considered rare if its prevalence is less than 1:2,000. The European Commission estimates a total of up to 8,000 rare disorders in Europe. Consequently, although individually rare, RD represent a significant public health problem affecting about 36 million people in EU. For the Croatian population, this means that 250,000 people may be affected by RD. RD are defined not only epidemiologically by their low prevalence, but also clinically as chronic, complex (i.e. affecting multiple organs or organ systems), life threatening and/or debilitating, often requiring long and expensive specialised treatments. These common characteristics led to the fact that they are addressed together at European level. The European Council adopted in June 2009 the *Recommendation on an action in the field of rare diseases*, supporting the adoption of national plans and strategies to guide and structure actions in RD within their health and social systems before 2013.

Multiple challenges in the field of RD were identified in a survey conducted by the Croatian Society of RD of the Croatian Medical Association and the Croatian Association of Patients with RD, during their joint meeting in Dubrovnik in 2010. Awareness of and common knowledge regarding RD are generally lacking. There are difficulties in the coordination of clinical management, shortcomings in the equipment and personnel of the centres of expertise, therapies are often very expensive and difficult to access. Deficits are present with respect to diagnostics, therapy, screening of rare diseases, as well as to research development. Due to the coding problems in hospital settings and the lack of registries, there is presently no valid information on the epidemiology of RD in Croatia.

The National programme for RD was developed in the following years by the dedicated Committee of the Ministry of Health and adopted by the Government, on 5 March 2015. It includes nine strategic areas of activity: • Improving the knowledge and availability of information on RD; • Supporting the development of registers of RD and their sustained funding; • Supporting the work and development of a network of reference centres and relevant scientific organizations for RD; • Improving the availability and quality of health care (diagnosis, treatment and prevention) for patients suffering from RD; • Ensuring the availability of drugs for RD; • Improving the implementation of social rights of people affected by RD; • Strengthening associations of patients with RD; • Promoting scientific research in the field of RD; • International networking and cooperation in the field of RD.

The Croatian National Programme for RD combines plan and strategy. For each field of action, objectives and according measures were defined based on the assessment of the current situation, to be fitted in the existing organisation of our system of care provision. The plan is the result of consultation process, associating experts in rare diseases, clinicians and researchers, representatives from patient groups, national health insurance, agency for medicinal products, and the ministries of health and of

research. A determined policy in this domain implicating all partners is needed in future in order to provide a response to the expectations of patients and their families.

Rare metabolic diseases in Croatia

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The term „Rare metabolic diseases“ refers to *inherited* metabolic diseases, i.e. those caused by inborn errors of metabolism. This group of disorders is characterized by a peculiar pathogenesis, which is specific by having an inherited biochemical disorder in the background. The primary cause is a mutation(s) of a gene, which results in the abnormal structure of the coded proteins, which can have different functions (enzymes, receptors, transporters, etc.). This leads to biochemical changes (lack of product and/or accumulation of substrate of metabolic reactions, inability of metabolites to cross cellular or intracellular membranes, etc.), which finally result in very variable clinical signs and symptoms. Inherited metabolic diseases belong the group of monogenic diseases, which are inherited either according to the Mendelian rules (majority) or maternally (those caused by mutations of mitochondrial DNA). To date, there are about 700 reported inherited metabolic disease, but their real number is much higher. Description of novel inherited metabolic disorders is almost continuous process with several new entities being reported every year. Despite impressive technology development in the last decades which has enabled quick simultaneous analysis of numerous samples for large number of metabolic diseases, on-time diagnosis as a precondition for successful treatment has remained one of the weak points of the health service for inherited metabolic diseases. It has been roughly estimated that about 1/3 of inborn errors of metabolism are completely treatable and 1/3 partly treatable. Efforts for developing new treatment options are largely directed by the interest of industry. Concerning inherited metabolic diseases in Croatia, the situation has many similarities to the global situation (average incidence of diseases, delayed diagnosis, limited experience with most inborn errors of metabolism, etc.), but also some specificities. Both are reflex of the economical situation, as well as of quality of health care organisation. In the latter sense many aspects could be improved. Awareness of the existence of inherited metabolic diseases as a whole should be increased at different levels, including ministry of health, Croatian health insurance institute, teaching institutions and health care providers at all levels of health care. Distribution of financial resources could be more even; while some groups of disorders are diagnostically and therapeutically properly supported, for instance lysosomal diseases due to the interest of pharmaceutical companies, the others are being neglected, for instance even those which should be diagnosed by extended neonatal screening program like organic acidurias, fatty acid oxidation disorders and some aminoacidopathies. Although centralized in Croatia, with predictable number of samples and expenditures, metabolic laboratory service suffers from shortage of personnel and equipment with subsequent relatively long turn around time making diagnostic work-up in many patients additionally problematic and costly. National drug lists could be more comprehensive in terms of having some drugs for metabolic diseases readily available, for instance vitamins for some vitamin-dependent inherited diseases. So called „interventional import“ for drugs which are not on national lists should be accelerated. A better educative/monitoring system,

perhaps a digital one, could be developed in order to decrease the number of tests which do not have proper indication. The activities related to the health service for inherited metabolic diseases should be organized and controlled by related referral centers which should be better supported by state institutions.

Voice of the patients: Rare but stronger together

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Why we need to hear voice of the patients? Patients are experts and payers. In spite of very different rare diagnosis patients face common problems. Those problems are:

- Lack of access to correct diagnosis
- Delay in diagnosis
- Lack of quality information on the disease
- Lack of scientific knowledge of the disease
- Heavy social consequences for patients
- Lack of appropriate quality healthcare
- Inequities and difficulties in access to treatment and care

How to overcome those problems:

- By implementing a comprehensive approach to rare disease
- By developing appropriate public health policies
- By increasing international cooperation in scientific research
- By gaining and sharing knowledge about all rare disease
- By facilitating the networking of patient groups to share their experience and best practices
- By providing comprehensive quality information to the rare disease community

Working together is the only way to quality care!

Lysosomal storage disorders: laboratory approach

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Lysosomal storage disorders (LSD) are a clinically heterogeneous group of more than 50 disorders. They are caused by enzyme, enzyme activator, membrane transporter or membrane protein defects that result in accumulation of complex macromolecules normally degraded in lysosomes. Individually they are rare, but this group as a whole has a prevalence of more than 1:7,000 live births. LSDs are always progressive, and often lead to severe symptoms and premature death. Patients present with a large phenotypic spectrum of disease manifestations that are generally not specific for LSDs, leading to considerable diagnostic delay and missed cases.

Over recent decades, considerable progress has been made in the treatment of LSDs and in patient outcome. The efficacy of many current and proposed therapies rely heavily upon early detection and treatment prior to the onset of irreversible pathology.

Newborn screening holds the promise of early detection. However, presymptomatic diagnosis raises a number of issues relating to patient management and treatment. We are, actually, still searching for "ideal" biomarkers for all LSDs. Clinical applications of current biomarkers (primary and secondary accumulating metabolites or proteins specifically secreted by storage cells) involve aiding diagnosis, monitoring disease progression, and assessing therapeutic efficacy.

LSD diagnostic strategy still mostly relies on initial clinical suspicion of individual symptoms that may occur from early infancy period to adulthood. Examination is usually followed by adequate specialist and laboratory management. Laboratory diagnosis of LSDs should be performed in several steps, depending on initial clinical symptoms. It most often begins with determination of specific metabolites in urine and/or serum and, depending on results, is followed by measurement of residual activity of lysosomal enzymes. Dried blood spot methods are currently available for identification of a range of LSDs. Molecular genetic testing is necessary to confirm the diagnosis of most LSDs and for diagnostics of X-linked diseases and pseudodeficiencies. Whenever genotype/phenotype correlations are available, they can be helpful in prognosis and in making decisions about therapy. Fast, reliable and affordable high throughput DNA sequencing, such as whole or selected exome sequencing, helps to make diagnosis in difficult cases and to reveal novel gene defects. Bioinformatics will be necessary to handle the data generated by these new technologies.

Due to clinical variety LSDs still represent a challenge in modern medicine so that it is of particular significance to use recommended integrative algorithms for diagnostics of treatable LSDs.

Diagnosis of Rare Metabolic Disorders in the Era of Next Generation Sequencing

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Inherited metabolic disorders represent important group of rare diseases. They refer to different types of medical conditions caused by genetic defects that interfere with metabolism. People with inherited metabolic disorders may have a variety of symptoms without a diagnosis, or they may have been misdiagnosed. Clinical evaluation and laboratory biochemical testing are usually used as tools for

metabolic disorders diagnosis. However, molecular genetic analysis is being increasingly applied, not only to confirm clinical and laboratory diagnosis of rare inherited metabolic disorders, but also as a tool for genetic counselling. The standard methods for genetic testing are Sanger DNA sequencing and multiplex ligation-dependent probe amplification (MLPA). A new generation of technologies, the so called “next generation sequencing” (NGS), offers a powerful approach and promises to reduce sequencing costs, to significantly increase the throughput, and to allow accurate sequencing of a large number of DNA sequences in a matter of days.

We have set the base for genetic testing and genetic counseling for several rare metabolic disorders combining targeted gene analyses (Sanger sequencing) with simultaneous analysis of 4813 genes (Clinical Exome NGS). We have reached >90% mutation detection rate. Among 75 analyzed patients with hyperphenylalaninemia, we have diagnosed 74 with phenylketonuria (variants detected in *PAH* gene) and 1 with tetrahydrobiopterin deficiency (variants detected in *PTS* gene). In the group of patients with glycogen storage diseases, we have diagnosed 24 GSD Ib (variants detected in *SLC37A4* gene), 2 GSD Ia (variants detected in *G6PC* gene) and one GSD III (variants detected in *AGL* gene) patient. The cohort of patients with branched-chain organic acidurias included 4 MMA (variants detected in *MUT* and *MMAA* genes), 4 MSUD (variants detected in *BCKDHA* and *BCKDHB* genes) and one 1 PA (variants detected in *PCCB* gene) patient. Clinical exome sequencing has enabled diagnosis of genetically heterogeneous diseases, such as mitochondriopathies (e.g. a patient with defect in the *SCO2* gene). Moreover, different diseases with overlapping clinical manifestations have been accurately diagnosed. Thus, pathogenic variants in the *PEX6* gene have revealed diagnosis of a defective peroxisomal biogenesis disorder instead of lysosomal storage disease, while pathogenic variants in the *SBDS* gene pointed to Shwachman–Diamond syndrome diagnosis after initial suspicion of GSD Ib.

Our approach leads to timely and accurate genetic testing, sets definite diagnosis and enables rapid implementation of optimal therapy for patients with rare metabolic disorders. Furthermore, we have provided the first molecular genetic data for several metabolic disorders for South-Eastern European region. In our study we have detected novel variants. For all genetic variants that had not been reported previously, we have performed *in silico* analysis and/or expressional *in vitro* analysis in order to assess their pathogenic effect. Thus, our data contributes to unambiguous diagnostic interpretation of novel genetic variants found in patients affected with rare metabolic disorders.

Genetic and clinical aspects of Fabry disease

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Fabry disease (FD) is an x-linked rare disease caused by pathogenic mutations affecting the *GLA* gene. That results in deficient activity of the lysosomal enzyme α -galactosidase A (α -Gal A) with a progressive accumulation of glycosphingolipids, the most prominent being globotriaosylceramide (Gb3), in various cells in the body.

The severity of the clinical phenotype in affected males is broadly related to residual α -Gal activity: the lower the enzyme activity, the earlier is the age of onset, and more severe and multi-systemic are the

clinical manifestations. In classic FD, caused by complete absence or marked deficiency of α -Gal activity, the vascular endothelium and smooth muscle cells, the peripheral and autonomic nervous systems, the kidneys, the heart and the brain are major sites of pathology, and the affected males usually become symptomatic during childhood or adolescence. In patients with higher levels of residual α -Gal activity, the resultant phenotypes are more organ-restricted, usually to the heart, and have a much later clinical onset, frequently in mid-adulthood. Hemizygous males typically experience the most severe manifestations of FD, while heterozygous females have symptoms ranging from very mild to severe as in men.

As patients present with a large phenotypic spectrum of disease manifestations that are generally not specific for FD, it is leading to considerable diagnostic delay and missed cases. Introduction of new disease modifying therapies for FD has made early diagnosis a priority. Increased awareness, but particularly the introduction of screening programs allow for early diagnosis and timely initiation of treatment.

Recently, as a consequence of systematic screening programs for FD in low and high risk populations, an unexpected high frequency of late onset and unknown significance genetic variants (GVUS) in the GLA gene have been reported. Because of the non-specific features of the late-onset cerebrovascular, cardiac and renal complications of FD, and the much higher prevalence of other causes of stroke, left ventricular hypertrophy and renal failure in adult populations, FD case-finding studies among high-risk patients are possibly intrinsically biased. This uncertainty often leads to considerable distress and inappropriate counselling and treatment. Accordingly, reports of patients identified in such studies, carrying either novel GLA sequence variants or GVUS, particularly when associated with high residual α -Gal activity, should provide enough clinical, biochemical and histopathological details to support the definite diagnosis of FD. When these features are non-specific, a definite diagnosis cannot be established and follow-up is indicated.

ERT should be considered only in those patients with a confirmed diagnosis of FD.

Fabry disease in children

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Fabry disease (FD) is a rare X-linked inborn error of the glycosphingolipid metabolism. Mutations of the *GLA* gene result in the deficient or absent lysosomal α -galactosidase A (α -Gal A) activity. More than 600 pathogenic mutations have been identified, most of which are private, occurring in individual families. Disease severity is inversely related to α -Gal A activity. As a result of deficient or absent α -Gal A activity, there is progressive accumulation of globotriaosylceramide and related neutral glycosphingolipids, within lysosomes of a wide variety of cells throughout the body. Glycosphingolipid storage results in a cascade of structural and functional cellular changes, triggering inflammation and fibrosis, and generally resulting in major organ dysfunction. Classically affected hemizygous males, with no residual α -Gal A activity, may display all the characteristic skin (angiokeratoma), kidney (proteinuria, renal failure), cardiac (cardiomyopathy, arrhythmia), neurological (neuropathic pain), and cerebrovascular (transient ischemic attacks, stroke) signs of the disease.

The first signs and symptoms of classic FD appear in childhood but diagnosis is often missed or delayed. The most common early features of FD include neurologic manifestations (acroparaesthesias, chronic neuropathic pain, hypohydrosis, anhidrosis, heat intolerance), tinnitus, hearing loss, gastrointestinal dysfunction (abdominal pain and diarrhea), angiokeratoma and ocular abnormalities (cornea verticillata, tortuous retinal vessels and subcapsular cataracts). Chronic fatigue and poor weight gain may also frequently occur. These may be seen in both boys and girls, but the onset in female heterozygotes is generally 2 to 5 years later and symptoms may be more variable. Although not life-threatening, these manifestations may have impact on quality of life of affected children. In addition, signs of major organ damage (microalbuminuria or proteinuria, urinary hyperfiltration, impaired heart rate variability, left ventricular hypertrophy, stroke) are encountered in children with FD.

Timely diagnosis is important as early treatment with enzyme replacement therapy reduces glycosphingolipid accumulation, alleviates symptom severity, can stabilize disease progression and, potentially, reverse underlying pathologic abnormalities. Physicians should be familiar with signs and symptoms of FD in childhood, to recognize the disease early in order to provide early access to treatment for their patients.

LOPD in EMG laboratory: Where to start? What to expect?

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Pompe disease (PD), also known as glycogen storage disease type II (GSD II) or acid maltase deficiency, is increasingly recognized as a cause of progressive muscle weakness in both pediatric and adult populations. In past, diagnosis required enzyme analysis in skin fibroblasts or muscle, but PD can now be readily diagnosed by analysis of enzyme activity in blood, using dried blood spot. The current challenge for clinicians is reaching the point at which PD is suspected, especially in adults. In this process of building a suspicion for late onset PD (LOPD) the clinical electromyoneurography (EMNG) could be a valuable tool.

Most of the data available on the electrodiagnostic abnormalities seen in PD were established from case reports published in the 1960's and 1970's. The most frequent finding was myotonic discharges without evidence of clinical myotonia. Sensitivity of EMG examination depends on the range of the diagnostic procedure and the muscles selected for EMG analysis. The specific complex repetitive discharges are expected to be found in patients with LOPD in paraspinal muscles. If the paraspinal muscles are not analysed, the sensitivity of EMG in LOPD diagnostics is significantly reduced. Spontaneous EMG activity in paraspinal muscles should be analysed in any patient with clinical or laboratory signs of myopathy, respiratory weakness of unknown cause, statin associated myopathy without improvement after discontinuation of statins, in patients with elevated creatin kinase levels of unknown cause and in patients with limb girdle muscle weakness. EMG in PD will show myopathic changes in almost all pediatric patients with PD, but some late onset patients may present with normal

EMG examination. The mixed myopathic and neuropathic pattern could be found in glycogen storage disease type III (Cori disease), but in PD the neuropathic is not typical finding.

The presence of complex repetitive discharges or myotonic discharges isolated in paraspinal muscles is not specific for PD but should raise a suspicion in the context of above mentioned clinical or laboratory findings. More sensitive quantitative EMG methods, muscle ultrasonography or MRI may be also valuable diagnostic tool for PD patients, especially for monitoring the response to enzyme replacement therapy.

Gaucher Disease – Diagnostic Challenge in the Internal Medicine

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Gaucher disease is a lysosomal storage disorder (LSD) resulting from pathologic build-up of undegradated storage materials in lysosomes. It is one of more than forty identified LSDs, multisystemic progressive and chronic diseases. There are 3 clinical subtypes of Gaucher disease, but almost 95% of Gaucher cases are of type 1 (GD1).

Overall population prevalence of GD1 is 1 :100 000, inheritance is autosomal recessive, so the both parents must be carriers of GD, and any forth child of these parents will have Gaucher disease.

Pathogenetically mutations in the acid beta-glucosidase gene caused a deficiency of acid beta-glucosidase (glucocerebrosidase), an enzyme that helps to break down glucosylceramide. In Gaucher disease patients glucosylceramide accumulates in the lysosome of certai cells primarily tissue macrophages.

Substrate engorged macrophage cells called also Gaucher cells accumulate in the affected organs leading to hepatomegaly, splenomegaly, hematologic disorders, bone and lung diseases.

For patients with GD1 life expectancy at birth is decreased by about 9 years. There are some additional symptoms and co-morbidities associated with Gaucher disease type 1 like neoplastic disorders (CLL, multirole myeloma, Hodgkin and non Hodgkin lymphoma, Parkinson disease, pulmonary hypertension.

Diagnosis can be made by a simple blood test, on the leukocytes (peripheral blood) or cultured fibroblasts. Diagnosis is very often delayed, and there are many cosequences in the form of complications like pathological bone fractures, liver diseases, chronic bone pain, severe sepsis. Signs and symptoms can be non-specific, resulting in a delay in diagnosis or misdiagnosis.

Treatment of disease can be divided in nonspecific care mostly for hematologic (iron and vitamin supplementation, transfusions) and skeletal (pain manngement, calcium supplementation) disease, and specific care with enzyme replacement therapy for GD1 based on enzyme preparations like imiglucerase. Enzyme replacement has effects on improvement of anaemia and thrombocytopenia,

reduction of hepatosplenomegaly, decreased incidence of bone crisis, decreased fatigue, increased growth in growth rearded children and many other effects.

Gaucher Disease and Cancer

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Gaucher disease (GD) is panethnic autosomal recessive lysosomal storage disorder with very heterogeneous clinical presentation. It is characterized by the accumulation of glucocerebroside in the macrophages of the spleen, liver, bone marrow and other organs. The hematological abnormalities (anemia, thrombocytopenia), enlarged spleen and liver and bone pathology are the predominant symptoms. GD is classified into three phenotypes and type 1 (adult form) is the most common one, without central nervous system involvement. GD is the most frequent and first lysosomal storage disease which can be successfully treated by enzyme replacement therapy.

There are several reports indicated an increased risk of hematological and solid neoplasms in patients with GD type 1, including multiple myeloma, non-Hodgkin lymphomas, chronic and acute leukemia, Hodgkin disease, hepatocellular carcinoma, lung cancer, glioblastoma, testicular carcinoma, and bone cancer. The risk is especially high for multiple myeloma (6 times higher than expected) which represents the conclusion of all clinical studies and reports. However, pathophysiological mechanisms of increased risk for cancer is still unknown. Several theories have been proposed, including immune dysregulation, augmented macrophage activation, hyperferritinemia, lysosomal dysfunction and endoplasmic reticulum stress.

Regarding the increased incidence of multiple myeloma, and maybe other malignancies, treating physicians should be familiar with this fact and closely monitor patients. Clarification of the mechanisms involved in predisposition to cancer in GD type 1 can provide new insights into disease pathophysiology and serve as a basis for more personalized management and monitoring of patients.

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