Newborn screening

Newborn screening tests include :

- umbilical cord blood congenital syphilis screening (compulsory)
- congenital cataract screening - by equipping the red reflex in the pupil with an ophthalmoscope (compulsory since 2005
- congenital deafness screening - hearing examination using the method of transiently evoked otoacoustic emissions (compulsory from 2012)
- neonatal laboratory screening from blood taken from the heel :
  - since 2022, screening for spinal muscular atrophy (SMA) and severe combined immunodeficiency disease (SCID) has been newly recommended - so far it is a pilot project, the examination is voluntary
- developmental dysplasia screening of the hip joint - examination of the hips by an orthopedist
- examination of congenital critical heart defects using pulse oximetry - comparison of SpO_2 on the right upper limb and lower limbs (optional)
- ultrasonographic examination of the kidneys (optional) .

In the narrower sense of the word, neonatal screening means **neonatal laboratory screening** .

Screening = systematic targeted search for a certain disease before its clinical manifestation in an effort to prevent its possible consequences in time

- according to the selected population in which the disorder is sought, we distinguish between *nationwide* screening (eg neonatal screening) and *selective* (in at-risk populations

- *conditions for the introduction of nationwide screening:*
  - the sought-after disorder is clinically diagnosable only at a time when irreversible damage to the endangered system has already occurred, or without its early determination, the life of the carrier of the disorder may be immediately endangered
  - the disorder has a sufficient incidence in the population
  - effective treatment is available
  - there is a screening test with high sensitivity and sufficient specificity
  - the test is reasonably cheap (favorable cost / benefit ratio)[1].

Newborn laboratory screening

All neonates born in the Czech Republic are subjected to neonatal laboratory screening (NLS) of congenital or hereditary diseases listed below by the method of taking a so-called dry drop of blood on a neonatal screening card between 48 and 72 hours of age. The goal of neonatal screening is rapid diagnosis and early treatment of newborns with these diseases.

- hyperphenylalaninemia and phenylketonuria ;
  - metoda: tandemová hmotnostní spektrometrie;
- congenital hypothyroidism ;
  - method: determination of thyroid stimulating hormone (TSH) by fluoroimmunoassay (FIA;
- congenital adrenal hyperplasia;
  - method: determination of 17alpha-OH-progesterone by immunoassay methods;;
- cystic fibrosis ;
  - method: determination of immunoreactive trypsinogen (IRT) level by immunoassay method
- selected inherited metabolic disorders:
  - method: tandem mass spectrometry
- spinal muscular atrophy and severe combined immunodeficiency (optional);
  - method: quantitative real-time polymerase chain reactions (QRT-PCR).

Methodology for collecting a dry drop of blood for the screening card

- well washed, perfused skin on the inner or outer edge of the newborn's heel is cleaned with alcohol and allowed to dry
- a small incision is made to a depth of max. 2 mm with a sterile spear by hand or lancet (special automatic device designed for this purpose
- the first drop of blood is wiped off with a dry sterile swab
- after creating a sufficiently large additional drop, the filter paper of the screening card is gently applied so that the blood is sucked up and completely fills the pre-printed target and the filter paper is visibly soaked on both sides.
  - the heel must not be squeezed or squeezed to prevent the admixture of tissue fluid
  - the target must be soaked from one drop at a time, the drops must not be layered into one target
  - it is necessary to soak up all the targets on the neonatal screening card with blood
  - we never touch the filter paper and it is necessary to avoid contact of blood drops with any object,

- after collection, let the blood dry in the horizontal position of the card for at least 3 hours at room temperature (preferably in a special card drying rack)
- after the blood has dried, the drops are covered with a cover paper, which is part of the card

Screening for hyperphenylalaninemia and phenylketonuria

- in the Czech Republic has been carried out since 1975, incidence according to NLS results (2010-2016) about 1: 5500[2]
- **hyperphenylalaninemia** (HPA) = a disorder of phenylalanine metabolism in which it pathologically accumulates in the blood and other body fluids → hyperphenylalaninemia **damages the CNS** - affects the mental development of the child and leads to severe oligophrenia
  - the most severe form: phenylketonuria (PKU) - incidence in the Czech Republic 1:10 000 live births
    - in 97% the cause is a **deficiency of the enzyme phenylalanine hydroxylase**
    - in1-3% the cause is **tetrahydrobiopterin** deficiency
- treatment (**elimination diet**) should be started within 21 days at the latest
- **screening** : *tandem mass spectrometry* method (since 1.10.2009)
- collection of dry blood drops from the heel of the newborn is performed only 3-4. the day of life on which the child receives the dairy diet (so that the phenylalanine level is high enough
- phenylalanine is present in approximately 5% of breast milk protein
- Criteria for the diagnosis of classical phenylketonuria
  - phenylalanine level above 20 mg / dl (above 1.2 mmol / l
  - normal or decreased tyrosine levels
  - the presence of abnormal metabolites in the urine (phenylpyruvic acid),
  - normal concentration of tetrahydrobiopterin, a phenylalanine hydroxylation cofactor to tyrosine

Screening for congenital hypothyroidism

- in the Czech Republic has been carried out since 1985, incidence in the Czech Republic according to NLS: about 1: 3000 live newborns
- **congenital hypothyroidism** (CH) - a lack of thyroid hormones in children leads to **impaired brain development** with a subsequent irreversible mental defect of varying degrees. Clinical symptoms appear late (only when the CNS is irreversibly affected)
  - it arises on the basis of thyroid **dysgenesis (agenesis, hypoplasia, ectopy) or dyshormonogenesis** (most often as a result of thyroid peroxidase deficiency); pituitary and hypothalamic forms are rare
- **L-thyroxine replacement therapy** should be initiated within 14 days of life
- **screening** : determination of *thyroid stimulating hormone (TSH) by fluoroimmunoassay (FIA)*
- All neonates with confirmed congenital hypothyroidism should undergo electronic screening for congenital hearing loss using transient otoacoustic emissions no later than 3 months after birth

Screening for congenital adrenal hyperplasia

thumb|CAH z deficitu 21-hydroxylázy.

- in the Czech Republic it has been carried out since 2006, the incidence of CAH is about 1: 8000 , according to the results of NLS about 1:13 000
- **congenital adrenal hyperplasia** (CAH) = a congenital disorder of steroid hormone synthesis caused by the absence of one of the five essential enzymes (most commonly **21-hydroxylase deficiency** and accumulation of 17alpha-OH-progesterone) resulting in increased production of adrenal androgens → **virilization** of female external genitalia, **premature pseudopuberty** (*pseudopubertas praecox*), short stature.
  - in 60% of those affected, a defect in mineralocorticoid production is also present
- **hydrocortisone replacement therapy (and mineralocorticoids)**
- **screening** : determination of *17alpha-OH-progesterone* by *immunoassay methods*

Screening for cystic fibrosis

- in the Czech Republic is carried out from 1 October 2009 , incidence according to NLS 1: 6500
- **cystic fibrosis** (CF) = AR inherited disease in which mutations in the gene encoding the chloride transporter on the cell membrane occur
- **screening**:

1. examination of the level of *immunoreactive trypsinogen (IRT) by immunoassay method*
2. if CF (high IRT) is suspected, the second step is a *molecular genetic analysis of the* most common, clearly pathogenic and population-significant mutations in the CFTR gene from the same dry blood droplets on the screening card in which an increased IRT concentration was detected
3. with very high IRT and no mutation, the third step is to perform a *sweat test* using pilocarpine iontophoresis.

Screening for other inherited metabolic disorders (DMP)

- **screening**: *tandem mass spectrometry* method .

Inherited disorders of amino acid metabolism

Organic aciduria (in NLS since 2009)

náhled|vpravo|Leucinóza (červeně místo poruchy

- **leucinosis (maple syrup disease, MSUD)** , incidence 1: 185,000 births
  - disorder of branched-chain amino acid metabolism (leucine, isoleucine and valine) → accumulation of toxic metabolites
  - clinical picture in newborns: food intolerance, failure to thrive, vomiting, lethargy and the smell of urine and earwax after maple syrup or caramel
  - without treatment it progresses to irreversible mental retardation, hyperactivity, failure to thrive, seizure disorder, coma, cerebral edema and can lead to death
- **glutaric aciduria type I (GA I)** , incidence 1: 40,000
  - glutaryl-CoA dehydrogenase deficiency, which converts glutaryl-CoA to crotonyl-CoA → increase in the level of toxic glutaric acid and its metabolites
  - clinical picture in neonates: macrocephaly but otherwise asymptomatic
  - later symptoms: metabolic acidosis, failure to thrive and sudden onset of dystonia and athetosis due to irreversible striatal damage

náhled|vpravo|Izovalerová acidurie (červeně místo poruchy)

- **argininemia (ARG)** , incidence 1: 300,000 births
  - leucine metabolism disorder - isovaleryl-CoA dehydrogenase disorder → specific metabolites that may be toxic accumulate
  - clinical picture in neonates: metabolic ketoacidosis, "sweaty feet", dehydration, hyperammonemia, ketonuria, vomiting, hypoglycaemia and failure to thrive; there are also milder forms without manifestations in the neonatal period

Urea cycle disorders (in NLS since 2016

náhled|vpravo|300px|Cyklus močoviny. CPS1: karbamoylfosfátsyntetáza, OTC: ornitintranskarbamyláza, ASS: argininsukcinátsyntetáza, ASL: argininsukcinátlyáza, ARG1: arginása.

- **argininémie (ARG)**, , incidence 1: 300,000 births;
  - arginase deficiency → accumulation of arginine and ammonia → irritability, eating disorders, vomiting, failure to thrive, neurological symptoms (clumsiness, walking on tiptoe around the age of 2-3 years, delayed psychomotor development, convulsions,...
  - hyperammonaemia attacks with increased amino acid breakdown (fever, starvation, infections, surgery) → ammonia is toxic to brain cells in high concentrations → confusion, impaired consciousness, vomiting; life threatening
- **type I citrulinaemia** (CIT), 2 patients have been diagnosed in the Czech Republic so far
  - argininosuccinate synthase deficiency → accumulation of citrulline and ammonia
  - severe neonatal form - very rapid progression with high mortality
  - late form - manifestations in situations of increased energy demands

Disorders of sulfur amino acid metabolism (in NLS since 2016

- **cystathionine beta-synthase (CBS) deficiency homocystinuria** , pyridoxine non-responsive form, incidence 1: 6000 - 1: 20,000
  - the most common disorder of sulfur amino acid metabolism, block in homocysteine transsulfurization
  - cystathionine beta-synthase deficiency → decreased condensation of homocysteine with serine to cystathionine → homocysteine and its derivatives accumulate
  - the enzyme cofactor is pyridoxal-5-phosphate, which is formed from vitamin B6 (pyridoxine) ingested in the diet
  - mild deficit: asymptomatic
  - severe deficiency: multisystem involvement of eyes, skeleton, central nervous system and blood vessels
  - pyridoxine treatment (some patients are non-responsive), a dietary methionine / protein restriction supplement supplemented with a cysteine-enriched preparation of essential amino acids and / or betaine
- **homocystinuria from methylenetetrahydrofolate reductase deficiency (MTHFR)** , incidence unknown
  - diseases of folate and sulfur amino acid metabolism disorders, the most common disorder in homocysteine remethylation
  - severe methylenetetrahydrofolate reductase deficiency → 5,10-methylenetetrahydrofolate is not reduced to 5-methyltetrahydrofolate → homocysteine and its derivatives accumulate
  - clinical picture of severe deficit: neurological symptoms (psychomotor developmental delay, hypotension and convulsions), later psychiatric problems, neuropathy and thromboembolic events
  - treatment: administration of high doses of betaine

Inherited disorders of fatty acid metabolism

Beta-oxidation disorders (in NLS since 2009)

- **medium chain fatty acid acyl-CoA dehydrogenase deficiency (MCAD deficiency)** , incidence 1: 20,000 births
  - beta-oxidation disorder of medium-length fatty acids → accumulation of fatty acids and their potentially toxic derivatives
  - starvation manifestations and / or periods of increased energy requirements (fever, stress), when energy production is largely dependent on fat metabolism

    - clinical picture in newborns: asymptomatic, event. hypoglycemia, metabolic acidosis, hyperammonemia and hepatomegaly, without treatment there is a high mortality, with treatment and prevention of hypoglycemia the prognosis is very good
- **long chain fatty acid dehydrogenase 3-hydroxyacyl-CoA deficiency (LCHAD deficiency)** , incidence 1: 60,000 births
    - clinical picture in neonates: attacks of Reye-like syndrome with cardiomyopathy and / or myopathy (rhabdomyolysis), hepatomegaly, hepatopathy, hypoketotic hypoglycemia, lactic acidosis and failure; even in well-treated patients, the clinical prognosis is uncertain
- **very long chain acyl-CoA dehydrogenase deficiency (VLCAD deficiency)** , incidence 1: 300,000 births
    - clinical picture in neonates with critical deficits: cardiomyopathy, arrhythmias, hepatopathy, rhabdomyolysis and sudden death

Beta-oxidation transport disorders (in NLS since 2009

- **carnitine palmitoyltransferase I (CPT I) deficiency**
    - beta-oxidation transport of long fatty acids; rare
    - clinical picture in neonates: asymptomatic or hypoketotic hypoglycemia, lethargy, hepatomegaly and convulsions, mostly caused by starvation or acute illness
    - treatment: a frequent diet (starvation prevention) high in starch-enriched carbohydrates and low in fat
- **carnitine palmitoyltransferase II (CPT II) deficiency**
    - beta-oxidation transport of long fatty acids; incidence unknown
    - classic adult form: episodic muscle weakness with myalgia, rhabdomyolysis and myoglobinuria, which are usually provoked by increased exertion, starvation, infection, stress or cold; the infantile form is very rare
- **carnitine acyl carnitine translocase (CACT) deficiency**
    - beta fatty acid transport disorder; rare

Hereditary vitamin conversion disorder

- **biotinidase deficiency (BTD)** - in NLS since 2016
    - organic acidemia ; incidence 1: 60,000 births
    - clinical picture according to the severity of the deficit: asymptomatic or life-threatening ketoacidosis, or gradual neurological and visual and hearing impairment

Spinal muscular atrophy and severe combined immunodeficiency

- pilot project since January 2022, examination is voluntary
- it is performed from a collected dry drop, there is no need to collect more blood
- real-time quantitative polymerase chain reaction (QRTPCR) screening

Newnatal Screening Laboratories

Laboratory examination **of hereditary metabolic disorders (DPM)** by tandem mass spectrometry is performed by:

- General University Hospital, Ke Karlovu 2, 128 08 Prague 2, Department of Hereditary Metabolic Disorders
- Olomouc University Hospital, IPPavlova, 775 20 Olomouc, Laboratory of Inherited Metabolic Disorders, OKBL

Laboratory examination of **congenital hypothyroidism (CH)** , **congenital adrenal hyperplasia (CAH)** and **cystic fibrosis (CF)** using immunoanalytical methods is performed by:

- Královské Vinohrady University Hospital, Šrobárova 50, 100 34 Prague 10, Laboratory of Neonatal Screening, Department of Children and Adolescents
- University Hospital Brno, Černopolní 9, 61300 Brno, workplace Children's Hospital, Department of Clinical Biochemistry and Hematology

Real-time laboratory testing of **SMA** and **SCID** by quantitative polymerase chain reaction:

- General University Hospital, Ke Karlovu 2, 128 08 Prague 2, Department of Hereditary Metabolic Disorders
- University Hospital Brno, Černopolní 9, 61300 Brno, Department of Internal Hematology and Oncology, Center for Molecular Biology and Genetics

Notes

- until 2009, the ***Guthrie test*** (semi-quantitative microbiological test) was used to determine **phenylalanine** in blood
    1. a drop of blood is taken on the target of the filter paper
    2. the filter paper is placed in an agar medium containing bacterial spores (usually Bacillus subtilis) and a competitive growth inhibitor specific for the amino acid sought (for phenylalanine beta-2-thienylalanine)
    3. incubation,
    4. evaluation of bacterial growth (bacteria grow in the presence of phenylalanine in a drop of blood)
- ferric chloride urine test was previously used

Template:Netisknout

LINKS :

https://www-wikiskripta-eu.translate.goog/w/Screening_d%C4%9Bdi%C4%8Dn%C3%BDch_chorob?_x_tr_sl=auto&_x_tr_tl=en&_x_tr_hl=cs (Screening for hereditary diseases)

https://www-wikiskripta-eu.translate.goog/w/Vrozen%C3%A9_vady_metabolismu_s_akutn%C3%AD_symptomatologi%C3%AD?_x_tr_sl=auto&_x_tr_tl=en&_x_tr_hl=cs (Congenital defects of metabolism with acute symptomatology)

Reference:

1. LEBL, Jan, Kamil PROVAZNÍK and Ludmila HEJCMANOVÁ, et al. *Preclinical pediatrics.* 2nd edition. Prague: Galén, 2007. pp. 183. ISBN 978-80-7262-438-6
2. LEBL, Jan, Kamil PROVAZNÍK and Ludmila HEJCMANOVÁ, et al. *Preclinical pediatrics.* 2nd edition. Prague: Galén, 2007. pp. 184. ISBN 978-80-7262-438-6 .\
3. JURÁSKOVÁ, Dana. *Bulletin of the Ministry of Health of the Czech Republic 6/2009* [online]. © 2009. Last revision 2009, [cited. 11. 12. 2009]. < https://udmp.lf1.cuni.cz/ns/ns_files/6_2009.pdf >.
4. http://www.novorozeneckyscreening.cz/vysledky-ns-2012-19-10-2015-131436
5. LEBL, Jan, Kamil PROVAZNÍK and Ludmila HEJCMANOVÁ, et al. *Preclinical pediatrics.* 2nd edition. Prague: Galén, 2007. pp. 185. ISBN 978-80-7262-438-6 .
6. COLLECTIVE WWW.NOVOROZENECKYSCREENING.CZ ,. *What is neonatal screening* [online]. [feeling. 2017-03-11]. < http://www.novorozeneckyscreening.cz/ov-co-je-novorozenecky-screening >.
7. Czech Republic. Bulletin of the Ministry of Health of the Czech Republic 14/2021. 2021. Also available from URL < https://www.mzcr.cz/wp-content/uploads/2021/12/Vestnik-MZ_14-2021.pdf >.
8. VÁVROVÁ, Jaroslava. *Nomenclature of the Ministry of Health of the Czech Republic* [online]. © 2008. Last revision 2008, [cited. 11. 12. 2009]. < http://ciselniky.dasta.mzcr.cz/hypertext/200640/hypertext/JVADB.htm >.

1. LEBL, Jan – PROVAZNÍK, Kamil – HEJCMANOVÁ, Ludmila, et al. *Preklinická pediatrie.* 2. edition. Praha : Galén, 2007. pp. 184. ISBN 978-80-7262-438-6.
2. http://www.novorozeneckyscreening.cz/vysledky-ns-2012-19-10-2015-131436