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CASE REPORTS AND MINIREVIEW

PRIMARY IMMUNODEFICIENCIES ASSOCIATED WITH DNA DAMAGE RESPONSE: COMPLEXITIES OF THE DIAGNOSIS

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Abstract

Introduction. Ataxia-telangiectasia (A-T) or Louis-Bar's syndrome and Nijmegen breakage syndrome (NBS) belong to primary immunodeficiencies associated with impaired DNA repair, because of the damage to genes involved in the repair system. The peculiarity of these orphan diseases is that the onset and development of clinical manifestations depend on the severity of genome instability, the rate of accumulation of mutations determining the severity of the course for each individual patient.
Cases presentation. The clinical cases of the syndrome A-T in a 4-year-old child and NBS in an 8-year-old child are presented in this article. The dynamics of clinical symptoms and their evolution were analyzed. The authors identified common clinical and immunological features of A-T and NBS in the examined children. The features of clinical cases were compared with data from the scientific literature.
Conclusions. The complexities of the A-T and NBS diagnosis, due to the peculiarities of the course in

Résumé

Immunodéficiences primaires associées à la réponse de l'altération de réparation de l'ADN : complexités du diagnostic

Introduction. L'ataxie-télangiectasie (A-T) ou le syndrome de Louis-Bar et le syndrome de rupture de Nimègue (NBS) font partie des immunodéficiences primaires associées à une altération de la réparation de l'ADN en raison d'endommagements des gènes impliqués dans le système de réparation. La particularité de ces maladies orphelines est que le début et le développement des manifestations cliniques dépendent de la gravité de l'instabilité du génome, du taux d'accumulation de mutations déterminant la gravité de l'évolution pour chaque patient.
Présentation des cas. Les cas cliniques du syndrome A-T chez l'enfant de 4 ans et NBS chez l'enfant de 8 ans sont représentés dans l'article. On a analysé la dynamique des symptômes cliniques et les caractéristiques de leur évolution, ce qui avait compliqué

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different patients and the variability of the onset of the first clinical manifestations, are highlighted. The analyzed clinical cases will contribute to better physicians' awareness and vigilance regarding the early diagnosis of primary immunodeficiency, that will prevent serious complications.

Keywords: ataxia-telangiectasia, Nijmegen breakage syndrome, DNA repair, primary immunodeficiency.

List of abbreviations:
AFP – alpha-fetoprotein
ATM – Ataxia-Telangiectasia Mutated
A-T – ataxia-telangiectasia
CBC – complete blood count
DNA – Deoxyribonucleic acid
DDR – DNA Damage Response
Ig – immunoglobulin
KREC – kappa-deleting recombination excision circles
MRI – magnetic resonance imaging
MRN complex – protein complex consisting of Mre11, Rad50 and Nbs1
NBS – Nijmegen breakage syndrome
PID – primary immunodeficiency
TREC – T-cell receptor excision circles

la vérification précoce du diagnostic. Les auteurs ont identifié les caractéristiques cliniques et immunologiques courantes de l'A-T et du NBS chez les enfants examinés. Les caractéristiques des cas cliniques ont été comparées aux données des sources de la littérature scientifique.

Conclusions. L'étude a montré la complexité du diagnostic A-T et NBS en raison des particularités du cours chez différents patients et de la variabilité de l'apparition des premières manifestations cliniques. Les cas cliniques analysés contribueront à une meilleure prise de conscience et vigilance des médecins quant au diagnostic précoce de l'immunodéficience primaire qui préviendra les complications graves.

Mots-clés: ataxie-télangiectasie, syndrome de rupture de Nimègue, réparation de l'ADN, immunodéficience primaire.

Introduction

Primary immunodeficiencies (PIDs) are a heterogeneous group of severe genetically-determined diseases, associated with damage to various parts of the immune system, manifested by disorders of adaptive or innate immunity[1-4]. Most PIDs have permanent changes in the genome of the human body, determining the further predicted course of the disease. There are PIDs associated with disorders of DNA repair, resulting in the accumulation of mutations in the child's body, which affect the further course of these diseases[5]. Ataxia-telangiectasia (A-T) or Louis-Bar's syndrome and Nijmegen breakage syndrome (NBS) belong to these PIDs. The direct cause of DNA repair disorders in both cases are mutations in genes that play a major role in the development of these diseases and, in particular, can not provide a repair of damaged DNA. The time of occurrence of certain clinical manifestations often depends on the nature and severity of negative exogenous factors, such as radiation and insolation, which cause different numbers of DNA mutations in each patient. Therefore, a feature of these PIDs is the unpredictable instability of the genome, which results in various patterns of immunodeficiency[5-7]. Both syndromes are characterized by specific phenotypic features, among which there are common symptoms: „café-au-lait" spots on skin, hypopigmentation of the skin, keratosis, increased risk of malignant neoplasms, physical developmental delay, recurrent infectious diseases of the respiratory tract, hypogonadism, hypersensitivity to ionizing radiation[8-11]. Both syndromes belong to combined immunodeficiencies with associated or syndromic features, which in most cases are manifested by leukopenia, lymphopenia with decreased CD3, CD4, and hypogammaglobulinemia with IgA, IgE, IgG deficiency[2,10]. Syndromes of DNA repair may manifest by other symptoms characteristic of PIDs, in particular autoimmune diseases, allergies[9,12], although rare in these diseases[13].

Knowledge of the early clinical manifestations and the course of PIDs associated with disorders of DNA repair is essential for both primary care physicians and physicians of narrow specialties, for a timely diagnosis and prevention of severe complications of these diseases[14-15], because the radiation exposure of the children with PID, including X-ray examination methods, should be limited. Magnetic resonance imaging (MRI) should be preferred.

First case presentation

A 4-year-old boy was admitted to hospital with complaints of morning stiffness, knee joints pain and swelling, refusal to walk, low-grade fever, gait

and coordination disturbance, delayed motor development. Gait disturbance and frequent falls were remarqued and ataxia was diagnosed at the age of one year. The severity of these symptoms progressed with time. The patient underwent a set of examinations at the age of 2.5 years, which revealed a decrease in muscle strength during electromyography. Brain changes on MRI, including cerebellar hypoplasia, were not detected. He was under observation of neurologists with the diagnosis of ataxia and muscle hypotonia syndromes. Family history was non-contributory. Mother denied cancer in the family.

Eye telangiectasia (Fig. 1), „café-au-lait" spots and hypopigmentation on the skin, flexor-extensor contracture of knees, movement restriction in hips, elbow joints and cervical spine, ataxia, dysarthria, severe muscle hypotonia were revealed at physical examination. Lymph nodes hypoplasia was detected. The patient's weight was 13.5 kg, weight for age z-score –2.3; height 102 cm, weight for age z-score –0.9.

Transient leucopenia (3700 cells/m^3) and constant lymphopenia (625-1800 cells/m^3) were detected in complete blood count (CBC). The serum alpha-fetoprotein (AFP) level was increased more than tenfold the upper limit of normal (104 IU/mL vs 9.96 IU/mL normal value).

The concentration of serum immunoglobulins (Ig) A and E, and the number of CD3, CD4, CD8, CD19, CD16/56 lymphocytes were decreased, while IgM level was increased (Table 1).

Ultrasound examination revealed signs of bilateral coxitis and bilateral knee bursitis.

Molecular genetic analysis of six common *ATM* gene mutations did not found these mutations. However, typical clinical data: ataxia, telangiectasia, and elevated AFP allow setting the diagnosis of A-T according to the European Society for Immunodeficiencies criteria for the clinical diagnosis of A-T[16] and Juvenile Idiopathic Arthritis. Further molecular genetic analysis of ATM gene panel confirmed the diagnosis of A-T.

Regular methotrexate therapy has resulted in the improvement of arthritis, but 3 months later the first episode of pneumonia was detected. Coughing, nasal discharge, continuous crackles in the lungs lasted for a long time. intravenous immunoglobulin replacement therapy was prescribed.

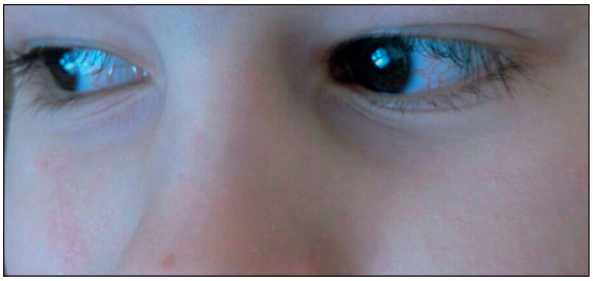

Figure 1. Ocular telangiectasia.

Second case presentation

An 8-year-old boy was admitted to hospital with complaints of frequent respiratory diseases, wet cough (productive cough), loss of appetite, physical retardation, intermittent pain around the umbilical region, a tendency to constipation.

This was the second pregnancy against background of acute respiratory infection at the 25-26 weeks of gestation, gestosis in the first trimester, placental insufficiency, oligohydramnios. The first child was healthy. From the family history, the maternal uncle died of pancreatic cancer. The premature birth was at 36 weeks of gestation. The child was born with a weight of 1320 g (more than –3 z-score weight for gestation age), length 39 cm (more than –3 z-score length for gestational age), and head circumference 29 cm (more than –3 z-score for this gestational age). The child's condition after birth was severe, with Apgar score 6/6 points. Resuscitation measures were performed, such as mechanical ventilation with an Ambu bag.

Up to 3 months the baby was under the supervision of the medical staff from the department of premature infants. He has lagged behind the neuropsychological development, but the indicators corresponded to the age norm up to one year. The child was periodically observed because of diarrhea, vomiting from birth, especially in the first months of life. Anthropometric indicators of the child's physical development throughout life have always been lower than the age norm.

After the 1st year of life, the child has often had recurrent respiratory infections (up to 20-28 times a year), with frequent antibiotic therapy. He had decreased appetite, a tendency to constipation, poor weight gain and height, anemia after the 2nd year of life. The boy was observed by a pediatrician, gastroenterologist. He was diagnosed with malabsorption syndrome and repeatedly examined by an endocrinologist and a genetician for delayed physical development. Cystic fibrosis and other causes of malabsorption have been suspected and were excluded, taking into account the frequent respiratory infections and low-weight. PID screening with CBC and determination of levels of immunoglobulins A, M, G have revealed only anemia (Hb 10.2 g/dL). At the age of 8 years, the child was examined for the presence of hereditary diseases of aminoacid metabolism or acylcarnitines, with negative results.

Table 1. Immunological parameters in observed patients

Parameter	*Case 1*	*Case 2*
Cells/m^3 (%)	4.5 years	8 years
Leukocytes	6000	7600
Neutrophils	3120	4330
Lymphocytes	1800*	2960
CD3	990* (55%)*	1390 (47%)*
CD4	470* (26%)*	947 (32%)*
CD8	410* (23%)	440* (15%)*
CD19	90* (5%)*	207* (7%)*
CD16/56	252 (14%)	355 (12%)
IgA, g/L	0.35*	1.38
IgM, g/L	2.76**	1.09
IgG, g/L	8.54	9.04
IgE, IU/mL	<0.1*	34

*-decreased level; **-increased level

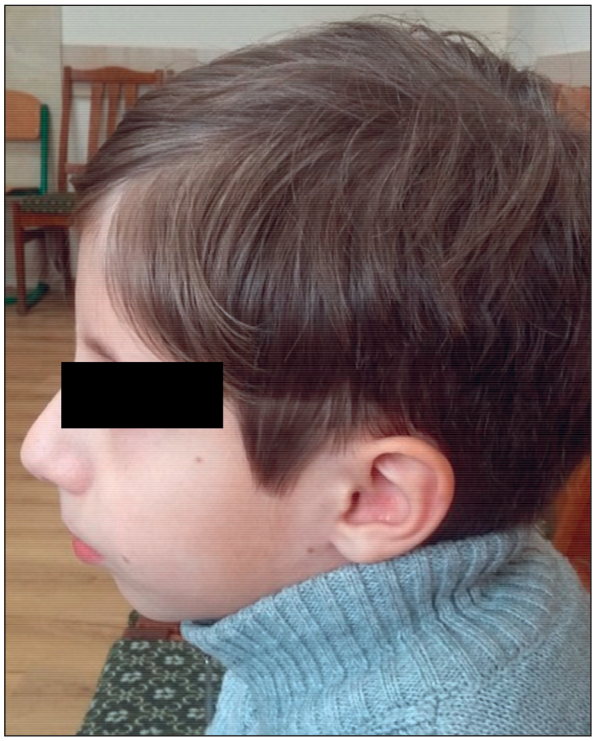

Figure 2. Dysmorphic features (a prominent nose, large ears, a small jaw in the patient with NBS).

The distinctive facial features: a prominent nose, large ears, a small jaw, a short neck, which were embedded in the phenotype of „bird-like" facies (Fig. 2). He lagged behind the physical development: his weight was 15 kg (weight for age *z*-scores –5.1), height 113 cm (height for age *z*-scores –2.71), head circumference 49 cm (5 percentile). Auscultation: a large number of dry whistling and small-bubble moist rales. The neuropsychological development and cognitive functions corresponded to age. Leukocyte and lymphocyte levels were normal.

The immunological study revealed moderate deviations of lymphocyte subpopulations CD3, CD4, CD8, CD19 (Table 1).

The NBS was suspected, considering congenital microcephaly, prenatal hypoplasia, facial dysmorphia, physical retardation. The molecular genetic study revealed mutation 657del5 ("Slavic mutation") of the *NBS1* gene, confirming the diagnosis. The boy was homozygous for this mutation, and the parents were heterozygous.

Discussion

At the base of the development of A-T syndrome and Nijmegen breakage syndrome are mutations in the genes involved in the DNA repair system[5-7].

DNA is the only macromolecule of the cells of the human body which has the ability to repair defects of its own structure through a set of mechanisms of repair regulation (DDR-complex, DNA Damage Response – response to DNA damage) and thus to maintain the stability of the cell genome[5-7]. The regulatory mechanisms breach of DNA repair leads to the accumulation of mutations, that can cause the development of immunodeficiency states, malignant, autoimmune and neurodegenerative diseases or their combinations, and contribute to increased sensitivity of the human body to exogenous factors (radioactive radiation, etc), which have a negative impact on the stability of the genome (initiation and accumulation of new mutations)[6-7].

The reason for the development of Louis-Bar's syndrome is a mutation of the ATM gene, leading to the breach processes of DNA repair. The ATM gene is localized on the long arm of chromosome 11 (locus 11q22-q23) and participates in the repair of double-stranded DNA damage, in the regulation of the cell cycle (meiosis), the apoptosis system, etc[6-7]. The accumulation of mutations due to disruption of DNA repair processes initiates damage of the chromosomes 7 and 14, on which the genes of the T-cell receptor and heavy chains of immunoglobulin molecules are localized[5,7,10]. Therefore, both cellular and humoral parts of immunity suffer (synthesis of antibodies, maturation of T- and B-lymphocytes are broken). Nowadays, more than 200 mutations leading to the development of this disease are known, and the A-T syndrome itself includes two variants of the course, which differ in the severity of the clinical picture, the time of manifestation, the severity of immunodeficiency[7,10]. A large variety of mutations of the ATM gene did not make it possible in the first genetic

study, which included only 6 mutations, to confirm the A-T syndrome in our patient.

There are also changes in the immune system (hypo- or aplasia of the thymus, lymph nodes and immunogram parameters that are typical for combined immunodeficiency) in patients with A-T syndrome, in addition to progressive cerebellar ataxia, telangiectasia (dilation of skin microvessels and sclera), „café-au-lait" spots, skin hypopigmentation, disorders of physical and neuropsychological development[5,10].

Our child's primary clinical symptoms of Louis-Bar's syndrome after the 1st year of age were progressive cerebellar ataxia and muscle weakness. Telangiectasia, „café-au-lait" spots, skin hypopigmentation, impaired physical and neuropsychological development (dysarthria, static and motor skills disorders) have appeared much later.

Lymphopenia is typical for A-T[2,10] in the neonates. Newborn screening of severe combined immunodeficiency, using the measurement of T-cell receptor excision circles (TREC) and kappa-deleting recombination excision circles (KREC), identifies severe T- and B-cell lymphopenia, alongside with making possible to find A-T babies right after birth[17]. Immunological abnormalities in A-T patients include declining amount and functional activity of T-lymphocytes, mostly due to CD4 and CD8, leading to an inadequate response to infectious agents, as well as to propensity for malignant neoplasms and auto immune processes, sharp reduction or lack in IgA, less commonly decrease or lack in IgG (IgG2, IgG4) and IgE subclasses, alongside with marked IgM increase. Besides, dysgammaglobulinaemia in the form of profound decrease in IgA, IgG, IgE[5,10,18] is possible. On the whole, our patient with A-T presented with low levels of IgA, IgE and high level of IgM.

A-T patients also presented with increased serum AFP[2,5,10,16], its level in the patient presented being 10.5 times higher than the normal value.

A-T clinical manifestations are very different in various patients, depending on the child's age, amount, and quality of systemic mutations, and intensity of exogenous damaging factors, so preventing prediction of the disease course in each specific case[5,7,10]. The early diagnosis problems in this particular case are most likely due to the lack of PIDs-indicating recurrent sinopulmonary infections in the onset of the disease. Late A-T diagnosis is noted by some authors[19-20].

The peculiarity of the case presented is that A-T has been suspected and diagnosed after the onset of autoimmune pathology, which is uncommon in A-T children. In particular, there's one available information on JIA in A-T children[13].

Such comorbidities require solving the drug therapy dilemma, since classical management of arthritis includes immunosuppression therapy, that is unacceptable for patients with immunodeficiency, leading to infectious complications, especially respiratory diseases. Among other risk factors are muscle weakness, frequent aspirations, poor muco-ciliary clearance, and initial immunological impairment. Regular medical examination, pulmonary function tests, immunology control and, if needed, intravenous immunoglobulin therapy can be helpful in diagnosing and treating respiratory complications, as well as in preventing development of chronic lung diseases.

This peculiar case indicates that A-T should be excluded in children with ataxia and muscle hypotonia, combined with physical and psychomotor disorders, and lymphopenia in CBC. No positive results of genetic and molecular test under restricted mutations determination is not a ground for A-T syndrome exclusion in case of typical clinical symptoms and elevated AFP level, according to European Society for Immunodeficiencies clinical criteria[16]. Commonly, cerebellum hypoplasia at MRI, another A-T criterion, appears later – at the age of 10 years[10]. Immunologic indices can also be helpful for the A-T diagnosis. ATM gene mutation diagnosis difficulties are due to the fact that most of A-T patients are compound heterozygotes, owing to a number of ATM gene versions[21].

Mutation of NBS1 gene, located on the 8th chromosome shoulder (locus 8q21)[5,7,22], is responsible for NBS development and impaired DNA repair. NBS1 gene encodes synthesis of nibrin protein, which is a part of MRN complex, involved in the regulation of DNA reparation (MRN complex activates ATM gene), and controls mitosis in mature lymphocytes[5,21,23]. More than 90% of the patients in both loci reveal a similar mutation of NBS1 gene, called 657del5 or „Slavic mutation", often found among the representatives of Slavic population in the Eastern Europe (Czech Republic, Poland, Ukraine)[22-23]. Besides, 11 other mutations of the gene, very rarely found in non-Slavic population (Germany, Canada, Italy, Mexico, Great Britain, the Netherlands, Russia)[21] have been described. Research findings within the framework of the International Project (1999-2009) aimed at determining the frequency of 657del5 „slavic mutation" in the newborns in Czech Republic, Poland, and Western Ukraine, confirming high NBS prevalence among the population, are of interest[23]. Particularly, in the Western Ukraine, the rate of homozygote mutant individuals was 1 per 34106 neonates, whereas each 95th newborn was a heterozygote carrier[23]. The patient presented in this paper lives in

the Western region of Ukraine, with high prevalence of the NBS1 gene „slavic mutation".

NBS and A-T have specific phenotype signs: microcephaly that progresses with age, „bird-like" facial appearance, growth delay, combined immune deficiency, often „café-au-lait" spots, skin hypopigmentation, telangiectasia[2,5,23]. In the case under discussion, the facial appearance typical for NBS was found at examination. Estimation of the boy's physical development revealed marked decrease in the body weight, indicating growth retardation that was assessed as somatogenic sub-nanism. In other authors' opinion, the most plausible causes of microcephaly and mental disorders are processes resembling immunoglobulin gene recombination in cerebral neurons ripening[22-24]. The presented child showed no signs of neuropsychological abnormalities, which are typical for the NBS children of the Western Ukraine[22].

Immunological changes, typical both for NBS and A-T, are characterized by moderate leukopenia, lymphopenia, decreased CD3, CD4, CD19 levels, as well as by decreased immunoglobulins, varying from isolated selective IgA and IgG (IgG2) subclass deficiency to hypogammaglobulinemia[5,22,23]. However, in NBS IgM level usually corresponds to reference values, sometimes being elevated. Besides, increased NK content is typical. These immune changes account for the high-risk of malignant neoplasms in NBS patients. In particular, the risk of lymphoma is 1000 times higher than the mean value for the population[25]. The patient under discussion had no leukopenia or decreased immunoglobulin levels. However, an insignificant decrease in the relative amount of CD3, CD4, CD8, CD19, and absolute amount of CD8 and CD19, complicating PIDs diagnosis, was found.

The intensity of the clinical manifestations of the Nijmegen syndrome varies among patients, depending, as in the case with A-T, on the number of mutations piled-up, age, and influence of adverse exogenous triggers[5,7]. Usually, microcephaly and characteristic facial appearance enable to diagnose the pathology in the first months postpartum[9,26]. In this particular case, the early diagnosis difficulties were due to prematurity, overlooked microcephaly in the premature baby, malabsorption dominating in the first years of life, that resulted in misinterpreting the causes of growth retardation and frequent infections, as well as lack of lymphopenia. This clinical case indicates that all babies with microcephaly should be examined for NBS.

The comparative characteristics of A-T and NBS are presented in Table 2.

Thus, the clinical cases presented highlight that syndromes of DNA repair disorder reveal themselves by different symptoms and abnormalities, rarely found in the children with this pathology. Lymphopenia, marked disturbances of immunological indices and recurrent infections may be absent. Nevertheless, the presence of the most significant criteria for diagnosing A-T (ataxia) and NBS (microcephaly) is ought to promote diagnostic efforts for

Table 2. Similar and different features of A-T and NBS

Characteristics	*A-T*	*NBS*
Mandatory criteria for the clinical diagnosis	Ataxia	Microcephaly
The most common characteristic features	Oculocutaneous telangiectasia	Typical facial appearance („bird-like" faces): a sloping forehead, prominent nose, large ears, small jaw, short neck
	Chronic, recurrent sinopulmonary infections	
Other typical features	Café-au-lait spots and/or hypopigmented areas and/or skin granulomas	
	Lymphoma/leukemia or other malignancies	
	Poor weight gain, growth delay	
	Hypogonadism	
	Lymphoid hypoplasia	Cerebellum hypoplasia on MRI
	Mental retardation	Late mild to moderate intellectual disability
	Radiation sensitivity	
	Chromosomal instability	
	Elevated alpha-fetoprotein	Normal alpha-fetoprotein
	Lymphopenia	
	Reduced T-cell number and function	
	Variable hypogammaglobulinemia, dysgammaglobulinemia: reduced IgA, IgG, subclasses G, IgE levels, elevated IgM level	

the purpose of excluding these diseases. Timely diagnosis is essential for the management of such patients. Adequate prevention of recurrent infections, avoidance of ionizing radiation are promising factors in terms of improving both life quality and expectancy of children with DNA repair disorder. The analysis of doctors, students, and postgraduate students' awareness revealed low knowledge about diagnostic algorithm in newborn microcephaly and lack of alertness for the detrimental effect of ionizing radiation in these syndromes[14,15,27]. Improvement of the doctors' awareness is expected to contribute to timely diagnosis and prevention of severe complications[8,28]. Neonatal screening of severe combined immunodeficiency is promising with regard to early diagnosis of these syndromes[17,29].

Conclusions

Diagnostic difficulties of DNA repair disorder syndromes are due to diversity and different period of clinical manifestations. Congenital microcephaly in Nijmegen breakage syndrome and ataxia in patients with ataxia-telangiectasia are the clue to suspect these diseases.

The clinical cases of A-T and NBS presented are expected to improve awareness and alertness of the doctors of different specialties in terms of early diagnosis of these diseases.

Author Contributions:

Y.B., O.B. was responsible for the diagnostic procedures, clinical diagnosis, and treatment decisions; M.K., O.B. wrote the manuscript, T.H., O.S. were responsible for the data acquisition, M.K., O.B., T.H. were responsible for the collection and assembly of the articles/published data, and their inclusion and interpretation in this review. All authors have read and agreed to the published version of the manuscript.

Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article"

„The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from the legal representatives of the patients included in the study."

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