Aims and Scope

The Annals of Diagnostic Paediatric Pathology is an international peer-reviewed journal. The focus of the journal is current progress in clinical paediatric pathology and surgical oncology in both basic and clinical applications. Experimental studies and clinical trials are accepted for publication, as are case reports supported by literature review. The main policy of the Annals is to publish papers that present practical knowledge that can be applied by clinicians.
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Genetics and biology of neuroblastoma - an overview

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Abstract

Neuroblastoma, the most common extra cranial solid tumor in early childhood, is a heterogeneous disease with different clinical and biological behavior. Unique features are the spontaneous regression and maturation which both require similar genetic prerequisites and, in the case of maturation, also the presence of the non-neoplastic Schwann cells. However, over the half of the tumors display an aggressive clinical course. Besides the stage and age at diagnosis, the MYCN oncogene (amplified versus non-amplified) has gained fundamental importance for therapy stratification, particularly in patients with localized disease and in infants. Further prognostic markers associated with an unfavorable outcome are DNA di-tetraploidy, deletion at chromosomal regions 1p36, 3p and 11q and gain of 17q, besides others. Most of these genetic markers have been shown to predict outcome in patients with localized disease while their impact in stage 4 patients is discussed controversially. Recent data indicate that not even MYCN amplification can separate prognostic subgroups in the latter patient group. However, a rapid BM-clearing seems to be associated with a far more favorable outcome even in the group of patients with unfavorable genetic features, thus expressing the response to chemotherapy and possibly providing an excellent prognostic marker. Furthermore, recent gene expression studies highlighting the whole transcriptome may provide also valuable information on the dignity of neuroblastic tumors. We can conclude that MYCN amplification still represents an excellent way to discriminate aggressive from non- or less aggressive tumors especially in patients with localized disease or infants. However, new biological and genetic information is necessary to be able to reach our final goal which is patient tailored therapy based on the genetic and biological profile of the tumor.

Key words: genetics, MYCN, oncogene

Genetic hallmarks in neuroblastic tumors

Over the last few years the amplification of the MYCN oncogene [11, 32] has taken shape as the most important marker for the assessment of the dignity of neuroblastic tumors. Amplifications of this oncogene can be observed in approximately 20% of tumors and are often associated with a deletion at the short arm of chromosome 1, del1p36 [10]. Both aberrations can be found in both genetic subgroups (Fig. 1) which are based on the different DNA-content (di-/tetraploid versus near-triploid or penta-/hexaploid). What up to now is entirely unclear is why MYCN amplifications and/or aberrations of the chromosomal region 1p36.3 occur in only about 15% of the near-triploid neuroblastic tumors while the same aberrations can be found in approx. 56% of the diploid group (unpublished data). The genetic aberration which is most frequently detected in neuroblastic tumors is gain of the long arm of chromosome 17 (in 66-69% of all cases) [9, 20, 39]. The prognostic value of this aberration has however not yet been completely clarified and was already opposed [36]. Also other chromosome aberrations like del11q23 [13, 15, 22, 35, 40] and del3p26 [13, 40] have been described frequently. Whereby, loss of sequences at 11q may indicate a rather promising genetic marker especially in MYCN non amplified tumors. However, one has to keep in mind that genetic/biological studies will only give clear results when the material to be tested is of good quality and is present in sufficient amount.

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Therefore, the sampling and storage of tumor, blood and bone marrow should be done in the appropriate way to guarantee the highest possible quality of the results [5]. Furthermore, quality assessment studies and a uniform nomenclature to describe the results of the genetic/biological studies provide additional security on the high quality of the results [1].

**Subgroups of neuroblastic tumors based on different DNA-content**

Based on a difference in DNA-content, neuroblastic tumors can be divided into two major groups (Fig. 1). Slightly more than half of the tumors (55%) have a near-triploid DNA-content (DNA-index between 1.26 and 1.74) while in approx. 45% of neuroblastic tumors a diploid (or tetraploid or even combined diploid/tetraploid) DNA content can be observed. Spontaneous regression or maturation, not triggered by therapy, and which can often be found in neuroblastic tumors, is very likely limited to the near-triploid subgroup [2, 7]. In approx. 15% of the cases, however, aggressive tumor behavior can also be observed in this subgroup (Ambros, unpublished). In these cases structural chromosome aberrations (MYCN amplification, 1p-deletion) may be found. Most of the diploid tumors, on the other hand, are aggressive even without MYCN amplification or 1p36.3 aberration, irrespective of the patient’s age at diagnosis or the stage of the tumor [19, 21]. Alterations of the genome can be observed in almost all diploid tumors.

**Genetic Origin of Neuroblastic Tumors and Genetic Intratumoral Heterogeneity (focal MYCN Amplification)**

When focusing on the two different ploidy groups, diploid versus near-triploid neuroblastic tumors, we can identify different frequency of structural chromosomal aberrations (i.e. deletions, gains or even amplifications). The majority of near-triploid tumors exclusively have only numerical chromosome aberrations – which seems to be sufficient for (often self-limited) tumor growth. Succeeding secondary changes, as for example MYCN-amplification or 1p36.3 aberration, lead, on the other hand, to malignant transformations (Fig. 1). As regards the diploid (2n) tumor group (without gain of additional chromosomes), the initiating event has not been identified so far, even though other chromosome aberrations like del11q23, del3p26 and 17q-gain have been described frequently [10]. What all these diploid tumors have in common, however, is their aggressive cell growth (Fig.1).

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**Figure 1**

Spontaneous regression and spontaneous maturation are restricted to those tumors with a near triploid (+ 3n) or near pentaploid/hexaploid (+5n/6n) DNA content. In tumors of patients under 1 year of age, lack of nerve growth factor (NGF) is a common finding [18, 27] and is probably involved in the phenomenon of spontaneous regression. Tumors undergoing spontaneous maturation processes, on the other hand, occur in patients over 1 year of age and are characterized by the presence of a reactive, i.e. non-neoplastic cell population, namely the Schwann cells, leading to a mixed population of near triploid (penta-, hexaploid) neuroblastic/ganglionic cells and diploid Schwann cells [2]. The tumor cells in both groups are usually characterized by whole chromosome gains, structural aberrations are found only occasionally. However, aggressive neuroblastomas frequently display diploid tumor cells, which can either show amplification of the MYCN oncogene and/or deletions at the short arm of chromosome 1, or display losses at the chromosomal regions 3p, 11q, 17q or other aberrations and high telomerase activity. 17q gain was found in both groups. Interestingly, also triploid tumors can acquire MYCN amplifications or 1p deletions, or other aberrations thus transforming this benign tumor into a highly aggressive one (dotted line). (MNA = MYCN amplification, LOH = loss of heterozygosity, NGF = nerve growth factor).

Figure modified from the article: ‘Neuroblastom’ by Peter F. Ambros and Inge M. Ambros in Medizinische Genetik 2:119-124 (2002)
Until recently, it has been assumed that the individual neuroblastotic tumors are genetically homogenous and that for this reason the investigation of a very small area of the tumor (e.g. biopsy) is genetically representative of the entire tumor. However, various facts led to quite different results. New and more sensitive techniques, i.e. serial genetic investigations of individual tumors used according to therapy-protocols and neuroblastoma screening, discovered also genetically in-homogenous tumors. Thus it could be shown that in up to 15% of all MYCN-amplified tumors the MYCN-amplification is focal, i.e. not ubiquitously detectable in the tumor tissue [4]. The discovery of the focal MYCN-amplification implies that this genetic change occurs only in the course of tumor progression – at least with some tumors – and causes, at least temporarily, heterogeneous cell populations within a tumor. It may furthermore be assumed that the amplified cells “overgrow” the non-amplified cells because of their advanced proliferation rate. These observations regarding heterogeneous tumors may be unexpected for neuroblastic tumors, but are not at all unusual in other tumor types in which amplified and non-amplified areas can be found side by side.

Since the first time that the familial neuroblastomas have been described in the middle of the last century, only a very few cases have been investigated and the involvement of the chromosomal region 16p seems to be important [24].

**Spontaneous tumor regression**

Complete spontaneous regression of tumors (even of “metastatized” tumors) without cytotoxic treatment is a phenomenon which scientists have not yet been able to explain. And even though spontaneous regression most often occurs in 4s stage tumors [12, 14], it is by no means limited to this stage. It can be observed in localised tumors [41] in 6 month old patients who have been diagnosed through the urinary mass screening and even in “classical” stage 4 tumors in the first year of life. In tumors which have the ability to regress spontaneously, near-triploidy (or polyploidy) was found but no MYCN-amplification or 1p-deletion [7] (Fig.1) was detected. The latter two genetic aberrations represent markers of aggressive tumor behavior. Moreover, the telomerase activity in aggressive neuroblastomas is increased as is the case in many other human neoplasias [16, 29]. 4s tumors and spontaneously regressing tumors however had low or absent telomerase activity. The only exceptions are patients with a stage 4s tumor and lethal outcome who did actually show an increase in telomerase activity [16, 29].

**Spontaneous tumor maturation**

Spontaneous tumor maturation (which is also not induced by cytotoxic treatment) does not occur in patients younger than 12 months but is observed in patients over one year of age. Fully matured tumors, i.e. ganglioneuromas, are usually only diagnosed in patients over 3 or 4 years of age. The genetic change consists of triplodization of the genome (or penta- or hexaploidization respectively) and a lack of chromosome 1p36 and MYCN-amplification [2]. In all ganglioneuroblastomas and ganglioneuromas a diploid cell population can be found besides a triploid cell population. Via in situ-hybridization it could then be shown that this diploid cell population is made up of Schwann cells which usually occur in mature tumors [2]. Until then, these Schwann cells, the amount of which increases during maturation, were considered to be tumor cells (like the ganglionic cells in the tumor) which led to the opinion that neuroblastic tumors evolve from “pluripotent” neural crest cell [37]. It is however, quite unlikely that a triploid neuroblast differentiates into a triploid ganglionic cell and a diploid Schwann cell. According to the current model [3], it is the neuroblastoma cell which, in the course of their cellular differentiation process, start to express chemotactic, mitogenic and also differentiation-inducing factors and thus “attract” Schwann cells, prompting them to proliferate and differentiate [23, 25, 26]. In addition, Schwann cells have an antiproliferative effect on NBs while at the same time stimulating differentiation [3, 4, 30, 34]. These findings confirm the central role of Schwann cells in the maturation process.

**Prognostic impact of bone marrow clearing in stage 4 patients**

Different approaches were used so far to investigate the prognostic value of tumor cell clearing in neuroblastoma patients. The techniques mainly used are based on immunological detection of GD2 stained cells using different detection systems. They range from conventional immunohistochemical detection assays [33], fluorescence microscopy [31] and a combined approach using fluorescence detection of the immunological target and subsequent FISH of unclear results [1, Modritz et al. in preparation]. Furthermore, RT-PCR was used to detect TH mRNA [17]. The different reports imply a similarity to the data in leukemia with their correlation between rapid bone marrow clearing and an increased probability of survival [28, 38]. However, we have to keep in mind that the ideal time point to obtain insights into the dynamics of the disease has to be determined in large multi-centre studies.

**References**


Neuroblastoma: histoprognostic classification and handling of material for biology - a challenge for the pathologist

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Abstract

Peripheral neuroblastic tumors originate from immature elements of the sympathetic nerve system and are characterized by a striking morphological, biological and clinical heterogeneity. For about 100 years, pathologists could not reach an agreement on a comprehensible and prognostic significant classification system. This paper summarizes the contributions made by Hiroyuki Shimada, who proposed to link morphological differentiation and cellular turn-over (measured as the number of mitotic and karyorrhectic nuclei) to the patients age, and describes the further development of his system into the International Neuroblastoma Pathology Classification (INPC) of 1999 with a recent revision of 2003. Pathologists play now a key role in the handling of neuroblastic tumors based on macroscopic inspection. They are responsible for an adequate sampling and saving of material for morphological, biological and genetic investigations, while the results of their microscopic assessment create the basis for a histoprognostic categorization beyond the subsets which presently are defined by biological/genetical markers.

Key words: classification, genetical markers, neuroblastoma, pathology

Introduction

Neuroblastomas belong, together with ganglioneuroblastomas and ganglioneuromas, to the group of peripheral neuroblastic tumors (pNTs) derived from sympatheticoblasts (sympathetic neuroblasts), i.e. immature precursors of the sympathetic nervous system.

They are considered as embryonic tumors originating either before or after birth from immature neural crest cells which are “left over” after normal organogenesis. Thus, the primary tumors are found in the same anatomical localization as their normal counterparts, i.e. in the adrenal medulla, and in sympathetic ganglia and paraganglia.

pNTs are the most common extracranial solid malignant tumors of childhood (1/10.000 births) and represent 15% of all malignant neoplasms occurring during the first four years of life [22]. 90% are diagnosed under the age of five years [18].

The challenge of heterogeneity

Since the sympathetic neuroblasts of embryonic origin display a restricted maturation capacity in individual tumors, pNTs are typified by an amazing heterogeneity, both with regard to the specter of histological phenotypes, biological properties and clinical behavior.

Already the very first reports which were published during the three last decades of the 19th and the beginning of the 20th century, outlined these neoplasms to comprise features of ganglioneuroma [16], neurocytoma/neuroblastoma [32] and ganglioneuroblastoma [21]. In 1934, Scherer reported on fully differentiated ganglioneuromas which contained nodules of either undifferentiated neuroblasts or differentiated ganglion cells, which he called “development centers” [23]. Thirteen years later, Stout confirmed these observations and suggested the designation “composite” ganglioneuromas/neuroblastomas for this variant of pNT which today would be categorized as nodular ganglioneuroblastoma [28].

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Biologically, subsets of pNTs, like neuroblastomas of stage 4S, can show complete spontaneous regression by massive apoptosis [10], while others, e.g. those with MYCN amplification, display aggressive proliferation and are frequently diagnosed at advanced stages of disease [4]. A third variant could be defined as those which grow slowly achieving more or less complete maturation. As early as in 1926, Cushing and Wolsbach proposed a sympathetic neuroblastoma to be able to transform into a ganglioneuroma after comparison of tumor material removed from the same patient with a time interval of 10 years [5]. This idea was later supported by a number of reports [6, 7, 12, 30]. Several authors pointed out that more mature lesions were associated with higher age of the patient [9, 31]. It is not difficult to understand that tumors of such great morphological and biological diversity will present with various clinical pictures.

**Histoprognostic classification of neuroblastic tumors**

Histopathological classification systems are designed to define categories and subtypes of diseases on the basis of commonly accepted and recognizable morphological features. To make them applicable to surgical pathology, these categories/subtypes should ideally be equivalent to clinicopathological entities implying essential clinical information about prognosis and appropriate treatment. Considering the above outlined heterogeneity of pNTs, it is obvious that a comprehensible histopathological classification system, based on reproducible morphological criteria and conveying valuable clinical information, is not created in a trice for these tumors.

*The dilemma of ganglioneuroblastomas.* Previous attempts to delimit prognostic categories of neuroblastomas and ganglioneuroblastomas, focused on the differentiation of neuroblasts and proposed to list parameters as nuclear and nucleolar size, relative amount of eosinophilic cytoplasm, and formation of clumps, rosettes and/or cytoplasmic (nerve) processes in an either quantitative [2] or qualitative manner [17]. Although ganglioneuromas were widely accepted as the most mature form within the specter of neuroblastic tumors, the relative amount of Schwannian stroma was not appreciated as a parameter of differentiation for less mature tumors like ganglioneuroblastomas before the early eighties of the 20th century. Nodules of undifferentiated neuroblasts or differentiated ganglion cells had indeed been recognized in ganglioneuroblastomas (see above), but rather vague designations as “development centers” or “composite” tumors indicated that the maturational relationship of these nodules to the surrounding ganglioneuromatous tissue was poorly understood. Some were even interpreted as a sign of focal partial or focal complete differentiation [2]. The search for a basic agreement to the entity of ganglioneuroblastoma was further hampered by a lack of commonly accepted histological criteria for this tumor: Some included neuroblastomas with a high degree of gangliocytic differentiation, while others required a substantial amount of ganglioneuromatous tissue between foci of differentiating neuroblasts [12]. Thus, a histoprognostic classification of ganglioneuroblastomas fell between two stools, i.e. immature neuroblastomas on one side and mature ganglioneuromas on the other.

**The Shimada Classification**

The credit for the first histoprognostical classification system which includes all peripheral neuroblastic tumors, goes to Hiroyuki Shimada [26].

*Histomorphological differentiation and MKI*

Shimada recognized the amount of Schwannian stroma in neuroblastic tumors as a major criterium to distinguish between immature (i.e. Schwannian stroma-poor) and mature (i.e. Schwannian stroma-rich) lesions.

The stroma-poor group was further subdivided into undifferentiated and differentiating tumors depending on whether the tumor contains less or more than 5% gangliocytic differentiated neuroblasts. This distinction represented a modification of the prognostic system originally proposed by Beckwith and Martin for neuroblastic tumors. As a measure of aggressive behavior of tumor cells in the stroma-poor group, Shimada proposed to analyse the mitosis-karyorrhexis index (MKI), i.e. the percentage of mitotic and karyorrhectic nuclei per 5000 tumor cells. He found that an MKI of <2% could be regarded as low, 2-4% as intermediate and >4% as high. Mitotic and karyorrhectic cells were summed up in one index because it was not always possible to distinguish between both phenomena on light microscopic grounds (Fig. 1).

The stroma-rich group contained three subgroups: (a) stroma-rich well differentiated tumors consisting mainly of ganglioneuromatous tissue with few immature neuroblastic cells, (b) stroma-rich intermixed tumors with nests of variably differentiated neuroblastic cells, and (c) stroma-rich nodular tumors with often grossly visible, hemorrhagic nodules of stroma-poor neuroblastoma displaying microscopically pushing borders towards the surrounding stroma-rich tissue. Shimada pointed out that his stroma-poor group overlaps with tumors called by others “classical neuroblastoma” and “diffuse ganglioneuroblastoma”, while the stroma-rich group corresponds to tumors previously called “ganglioneuroblastoma” and “immature ganglioneuroma”.

![Fig 1 Undifferentiated neuroblastoma with area of high MKI. Karyorrhectic cells often show condensed eosinophilic cytoplasm and hyperchromatic, fragmented nuclei. Round nuclei with a regular outer border might be lymphocytes and are not accepted as karyorrhectic.](image-url)
In his original paper of 1984, Shimada applied these morphological criteria on histological sections from 295 neuroblastomas and ganglioneuroblastomas recruited from two American, one Japanese and one British center. When looking at the distribution of the stroma-poor and stroma-rich groups and subgroups according to the patients age at diagnosis, he found that stroma-rich tumors had their debut at a relatively higher age, i.e. the stroma-rich intermixed and nodular ones mainly between two and five years, and stroma-rich well differentiated ones at the age of five years and older. These findings confirmed previous observations (see above) and supported the idea that the immature elements in the non-aggressive variants of these embryonic tumors have the capability to mature into benign ganglioneuromas over a certain number of years.

On the other hand, a disturbance (e.g. delay) of this “normal” sequence of maturation could probably forebode a tumor which because of its restricted maturation capacity will persist at an immature, biologically more aggressive stage. Shimada found strong support also for this hypothesis when he compared the age distribution of patients with stroma-poor tumors to MKI and clinical outcome. The majority of undifferentiated and differentiated stroma-poor tumors with low MKI diagnosed under the age of 1.5 year had a favorable outcome, while almost all undifferentiated tumors with low MKI diagnosed at >1.5 years killed the patients. Stroma-poor tumors with high MKI had a bad outcome, and the majority occurred in children older than 1.5 year. All children with stroma-poor tumors diagnosed at >5 years died.

The stroma-rich intermixed and well differentiated subgroups occurred in children older than 2 and 5 years, respectively, and almost all showed a favorable outcome. In contrast, most of the stroma-rich nodular tumors had an unfavorable prognosis, irrespective of their occurrence at the “correct” age of 2-5 years. It seemed probable that the stroma-poor nodules could represent a malignant outgrowth of biologically more aggressive clones within an otherwise maturing stroma-rich tumor.

Taken together, Shimada was the first to prove the usefulness of a classification based on a combination of histomorphology (i.e. the relative amount of Schwannian stroma and gangliocytic differentiation), MKI and age at diagnosis and concluded that

“1) the prognosis of the patients under 1.5 years old is directly influenced by the MKI regardless of the degree of maturation,
2) the prognosis of the patients between 1.5 and 5 years old is influenced both by the degree of maturation and the MKI,
3) the prognosis of the patients over 5 years old is poor regardless of the degree of maturation and/or the MKI.”

Modifications of the Shimada classification

In 1992, Joshi [12] searched to combine the conventional (pre-Shimada) terminology of neuroblastic tumors with the prognostic categories of the Shimada classification and suggested the following changes/additions:

1. The stroma-poor tumors of Shimada should be called neuroblastomas and defined as tumors with a neuroblastic component of >50% of the total tumor area.
2. Shimadas stroma-poor differentiated tumors should be splitted up into poorly differentiated neuroblastomas (containing neuropil and <5% differentiated neuroblasts) and differentiated neuroblastomas (containing >5% differentiated neuroblasts).
3. Shimadas stroma-rich tumors should be called ganglioneuroblastoma, nodular, intermixed and border-line and should be defined as tumors containing >50% ganglioneuromatous tissue. The borderline type was meant to replace Shimadas stroma-rich well differentiated type.
4. Tumors with equal amounts of neuroblastic and ganglioneuromatous components should be called “transitional neuroblastic tumors”. The term “unclassifiable neuroblastic tumor” should be reserved for tumors where the histological assessment was hampered by necrosis, hemorrhage, calcification, crush artifacts, cystic changes, and poor fixation/processing.

Interestingly, Joshi also performed a histop prognostic categorization of the nodules in nine nodular ganglioneuroblastomas (see below), without being able to show any significant impact on the survival of these few patients.

In other publications, he proposed a histological grading system based on the presence/absence of calcification and level of mitotic rate (MR) [13] which he later modified by replacing MR with MKI [15]. By combining these (modified) histological grades with the patients age, he defined (modified) low and high risk groups independent of the morphological differentiation.

The International Neuroblastoma Pathology Classification (INPC)

In 1994, the International Neuroblastoma Pathology Committee was established with the goal to achieve a “standardization of terminology and morphologic criteria of neuroblast tumors and establishment of a morphologic classification that is prognostically significant, biologically relevant, and reproducible” [24]. Both Shimada and Joshi were members of this committee, and the proposed classification represents by and large the original Shimada system of 1984 with a few modifications, some proposed previously by Joshi (see above), and some performed by the committee.

Morphological categorization

The following categories and subtypes of peripheral neuroblastic tumors were listed:

1. Neuroblastoma (Schwannian Stroma-Poor)
   Definition: PNT with 50% or less Schwannian stroma
   a. undifferentiated subtype
      consists of small-to-medium sized neuroblasts without neuritic processes (neuropil) or gangliocytic differentiation (Fig. 2). Scattered or foci of large or pleomorphic-anaplastic cells occur infrequently.
The diagnosis of this subtype of pNT may depend on ancillary techniques as e.g. immunohistology (NSE, CD56, tyrosine hydroxylase, synaptophysin, chromogranin A and markers to exclude myogenic tumors, primitive neuroectodermal tumors/Ewing sarcoma and lymphomas), cytogenetics and elevated urinary levels of metabolites of catecholamine synthesis.

b. poorly differentiated subtype

- consists of neuroblasts and neuropil. Less than 5% of neuroblasts show gangliocytic differentiation (Fig. 3).
- Scattered or foci of large or pleomorphic-anaplastic cells occur infrequently.

c. differentiating subtype

- consists of neuroblasts and neuropil. 5% or more of neuroblasts show synchronous gangliocytic differentiation (Fig. 4), i.e. enlarged, eccentric nucleus, vesicular chromatin pattern, prominent nucleolus and increased amount of cytoplasm (total cell diameter larger than twice the nuclear diameter). Areas of Schwannian stroma can be seen at the periphery of tumor (transitional zone), but do not exceed 50% of the tumor area.

2. Ganglioneuroblastoma, intermixed (Schwannian Stroma-Rich)

Definition: pNT with >50% ganglioneuromatous tissue which includes sharply defined nests of neuroblastic tissue (Fig. 5) containing neuropil and a mixture of differentiating neuroblasts and maturing ganglion cells (Fig. 6).

3. Ganglioneuroma (Schwannian Stroma-Dominant)

a. Ganglioneuroma, maturing subtype

- consists of >50% ganglioneuromatous tissue with scattered differentiating neuroblasts, maturing and mature ganglion cells which never lie in sharply demarcated nests (Fig. 7). This subtype corresponds to Shimadas stroma-rich, well differentiated [26] and Joshis ganglioneuroblastoma, borderline.

b. Ganglioneuroma, mature subtype

- consists of mature Schwannian stroma with fascicular neuritic processes and mature ganglion cells (Fig. 8). Neuroblasts are no longer present.
4. Ganglioneuroblastoma, Nodular (Composite Schwannian Stroma-Rich/Stroma-Dominant and Stroma-Poor)

Definition: pNT with a stroma-rich (like in ganglioneuroblastoma, intermixed) or stroma-dominant (like in ganglioneuroma, maturing) component which surrounds macroscopically visible, often hemorrhagic nodules of a neuroblastic, stroma-poor component (Fig. 9 a and b), regarded as a more aggressive clone. The nodule(s) show either pushing borders (Fig. 10) or a more infiltrative pattern towards the stroma-rich component. It is important to be aware of the possibility that a surgical biopsy may only contain a small rim of stroma-rich tissue around the nodule, i.e. the stroma-rich part will not necessarily make up >50% of the visible tumor area.

5. Terminology for not completely classifiable pNTs

a. Neuroblastoma (Schwannian Stroma-Poor), NOS is reserved for tumors which clearly belong to the category of Schwannian stroma-poor pNT, but which cannot be allocated to any subtype because of poor quality of the sections, bleeding, cystic degeneration, necrosis, crush artifact or diffuse calcification.

b. Ganglioneuroblastoma, NOS is applied to a stroma-rich tumor which extensive calcification which may obscure a stroma-poor nodule.

c. Neuroblastic tumor, unclassifiable is recommended for a pNT which cannot be allocated to any of the categories listed above. This may e.g. apply to very small biopsies.

Prognostic categorization

In order to (1) test the consensus between the committee members to recognize the above listed morphological categories and (2) identify the most powerful system for prognostic categorization, the committee reviewed 227 cases of pNTs [25] and compared the original Shimada system (based on morphology, MKI and age) with Joshi’s histological grades (based on MR and calcification), risk groups (based on histological grade and age) and their modifications replacing MR with MKI, which can only be used for prognostic analysis of stroma-poor tumors. The combination of morphology, MKI and age, originally proposed by Shimada, had the strongest impact on prognosis, measured as event free survival (EFS). The statistical analysis also confirmed the age of 1.5 years as the borderline of greatest prognostic significance. Beyond the age of 5 years, all stroma-poor tumors had a bad outcome.

Consequently, the International Neuroblastoma Pathology Committee included age and MKI, but not MR and calcification as relevant prognostic data for stroma-poor tumors into the new classification.

The MKI can be determined applying a method previously proposed by Joshi [14]. It is based on the initial estimation of cell density in different areas of a given tumor (Low: 100-300, Moderate: 300-600, High: 600-900 cells per high power field (HPF) of 400x magnification) and the subsequent definition of the number HPFs which are required to evaluate 5000 tumor cells. The MKI is reported as one of three classes (low, intermediate, high) according to the definitions originally given by Shimada [26].
Table 1 shows the prognostic allocation of the morphological categories and subtypes of pNTs as recommended in the INPC.

The process of reaching a final histoprognostic conclusion according to differentiation, MKI and age may appear confusing to the inexperienced. For newcomers, it can be recommended to learn the favorable categories by heart because their number is quite low: poorly differentiated and differentiating tumors with low and intermediate MKI below 1.5 years, and differentiating neuroblastoma with low MKI between 1.5 and 5 years. All other neuroblastomas are actually unfavorable.

Similar to the Shimada classification of 1984, Schwannian stroma-rich and dominant tumors have a favorable prognosis unrelated to the patient’s age, except for nodular ganglioneuroblastomas which all are ascribed an unfavorable outcome.

Revision of the International Neuroblastoma Pathology Classification (INPC)

Both Joshi [12] and the International Neuroblastoma Pathology Committee [24, 25] mentioned the possibility of performing a prognostic grading of nodular ganglioneuroblastoma (GNBn) on the basis of the stroma-poor nodule(s) since this component of the tumor had been regarded as the malignant one already many years ago [28]. However, the number of GNBns in their reviews was too small.

Umehara et al. [29] were the first to review 70 GNBns and to perform a prognostic categorization by analyzing the nodule(s) according to the above detailed criteria of the INPC for neuroblastomatous tumors (morphology and MKI) and relating their data to the patients’ age. These authors also described GNBns with single or multiple nodules and variant forms which (1) consisted mainly of the stroma-poor component surrounded only by a thin, microscopically visible rim of stroma-rich/-dominant tissue, and (2) only showed ganglioneuromatous tissue in the primary tumor (probably due to poor sampling), but a neuroblastomatous component at the metastatic site. Depending on the presence of nodule(s) of the favorable or unfavorable category, favorable (32.4%) and unfavorable (67.7%) subsets of GNBn were established which showed statistically significant differences in event free survival and survival, quite similar to neuroblastic tumors.

In 2003, The International Neuroblastoma Pathology Committee reviewed the same cohort of tumors and confirmed these results [20]. A revision of the INPC was proposed including the following terms: GNBn, classic, containing one nodule; and variant forms containing either multiple nodules, large nodules or no nodule, but a metastatic stroma-poor tumor. Unfavorable prognosis was defined by the demonstration of at least one nodule displaying features of the unfavorable INPC categories of neuroblastomatous tumors.

The acceptance of a large nodule as a variant of GNBn touches on one of the principles of the former INPC, which claimed that a tumor belonging to the stroma-rich or -dominant category has to be made up by >50% Schwannian stroma (see above). It is important for pathologists to be aware of all variants of GNBn because they were two times more frequent than the classic form among the cases reviewed by Umehara and Peuchmaur.

The greatest advantage of this INPC revision, however, is brought by the recognition of a subset (about 30%) of nodular ganglioneuroblastomas which, in contrast to previous thinking, belong to a prognostic favorable category which might benefit from a milder therapeutic regime.
Table 1
International Neuroblastoma Pathology Classification (INPC). Assignment of morphological categories/subtypes to prognostic categories according to MKI and age (modified from Shimada et al. [25])

<table>
<thead>
<tr>
<th>Category</th>
<th>Schwannian stroma-poor</th>
<th>Schwannian stroma-rich</th>
<th>Schwannian stroma-dominant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroblastoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Favorable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1.5 yrs</td>
<td>poorly differentiated or differentiating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5-5 yrs</td>
<td>&amp; low or intermediate MKI tumor</td>
<td>differentiating and low MKI tumor</td>
<td></td>
</tr>
<tr>
<td>Unfavorable</td>
<td></td>
<td>a. undifferentiated tumor</td>
<td>Schwannian stroma-rich</td>
</tr>
<tr>
<td>&lt; 1.5 yrs</td>
<td></td>
<td>b. high MKI tumor</td>
<td></td>
</tr>
<tr>
<td>1.5-5 yrs</td>
<td>a. undifferentiated or poorly differentiated tumor</td>
<td></td>
<td>Schwannian stroma-dominant</td>
</tr>
<tr>
<td>&gt; 5 yrs</td>
<td></td>
<td>b. intermediate or high MKI tumor</td>
<td></td>
</tr>
<tr>
<td>Ganglioneuroblastoma, intermixed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Favorable independent from age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ganglioneuroma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maturing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mature</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Favorable independent from age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ganglioneuroblastoma, nodular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite Schwannian stroma-rich/</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>stroma-dominant and stroma-poor</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 11 shows the morphologic and prognostic categories of the revised INPC.

Handling of tumor material for biological investigations
Independent from the above outlined endeavor of pathologists towards a clinically informative histoprognostic classification system for pNTs, a growing number of biological and genetic alterations has been discovered in these tumors during the past two decades which today allow an assignment to low-, intermediate- and high-risk groups [3]. The prognostic most important ones are still MYCN/1p status and ploidy. It is not possible to enter a child with a neuroblastic tumor into any of the current European clinical protocols without at least knowledge about MYCN, analyzed by Southern blot and/or fluorescence in situ hybridization (FISH).

Since many of these investigations either require or are most easily performed on unfixed tumor tissue, additional tasks have to be assumed by pathologists who usually are the first in the hospital to receive fresh tumor tissue from the operating theatre.

The various procedures for handling samples of neuroblastic tumors were in detail described by Ambros PF and Ambros IM [1], and will not be repeated here. The most central functions of the pathologist can be summed up in the following three key words: sampling, saving and microscopical evaluation.

Sampling. Taking into account the great morphological heterogeneity of these tumors, it is obvious that the pathologist, prior to any fixation, should cut the tumor into slices searching for e.g. nodules or hemorrhagic areas. It is important to take samples from every macroscopic variant, and this should proceed under sterile conditions if some of the material is planned to be used for culturing.

The further sub-sampling of any macroscopic area, being of interest for special investigations, follows a back-to-back principle, i.e. a representative piece designated for paraffin embedding should be used to produce at least 10 imprints from a fresh cut surface (for e.g. FISH or static ploidy analysis) before fixation in formalin. The two most closely neighboried (“opposite”) tissue slices should be snap frozen (for e.g. extraction of DNA, RNA and proteins, immunohistochemistry requiring unfixed tissue and interphase cytogenetics if imprints were not freshly taken) and put into a sterile culture medium (for e.g flow cytometry, conventional cytogenetics and culturing), respectively.

Saving. The adequate saving of the samples depends on the individual pathologists knowledge about the techniques which are required for the most essential biological investigations. Thus, imprints should be air dried overnight and then kept frozen in air-tight containers at -80°. Snap frozen tissue should also be stored at -80°, or in liquid nitrogen if preservation of RNA is intended. Frozen tissue cannot be used for conventional cytogenetics. Tissue planned to be analyzed by cytogenetics and flow cytometry, should be brought as soon as possible in phosphate buffered saline or culture medium to analysis, although it is possible to recover nuclei for FISH and flow cytometry from paraffin embedded tissue if fresh material is not available.
Microscopical evaluation. The pathologist should not only analyze morphology and MKI for the histoprognostic classification, but also use the microscope for estimation of the tumor cell content in the material designed for biological investigations. Thus, microscoping a paraffin section from the tissue sample which was used for imprinting prior to fixation, will provide important information about the possible percentage of tumor cells, non-tumor cells (e.g. lymphocytes or stroma) and necrosis which might influence the representativity of the imprint. A microscopic analysis of the tumor cell content performed on frozen sections from the material which is intended to be used e.g. for PCR of the 1p36.3 region, is even more demanded because the correct interpretation of the PCR result requires a tumor cell content of at least 70%.

It is obvious that the pathologist who is in charge of handling samples from neuroblastic tumors, plays a central role in providing adequate material for biological analyses.

Prognostic categorization by INPC and biological markers - competing or complementary systems?

It has been known for many years that MYCN amplification in neuroblastomas is a strong indicator of aggressive tumor behavior [4], and often associated with undifferentiated morphology and increased MKI [27]. One might therefore be tempted to ask whether the histoprog nostic categorization provided by the INPC could be replaced by the analysis of MYCN.

The correlation between INPC categorization and MYCN status was investigated by Goto et al. [8] in a large cohort of 628 patients with neuroblastic tumors. As expected, 97.7% of tumors with favorable histology (FH) were MYCN non-amplified, while 93.2% of MYCN amplified tumors showed an unfavorable histology (UH). However, the comparison of MYCN status and INPC categories revealed a third and surprisingly large group of tumors with UH but without MYCN amplification. As shown in Fig. 12, the prognosis of this group was intermediate to FH/non-amplified and UH/amplified tumors, and the difference in EFS between all three groups was statistically significant.

The majority of UH, non-amplified tumors were observed at stage 3 and 4 in children older than one year. The results of this investigation demonstrate that the histoprognostic INPC categorization is able to identify a substantial number of patients who, in spite of normal MYCN status, belong to a group of inferior prognosis, and it appears relevant to ask whether this category of patients should be offered a modified form of therapy.

Further support to this notion comes from the recently completed histopathological review of 124 stage 2A and 2B MYCN non-amplified trial patients who were treated with surgery alone according to the LNESG (Localised Neuroblastoma European Study Group) protocol 94.01 (Navarro et al., in preparation). The review was performed according to the INPC by the seven members of the SIOP European Neuroblastoma (SIOPEN) Pathology Reference Panel (Gabriele Amann, Klaus Beiske, Catherine Cullinane, Emanuele D’Amore, Claudio Gambini, Sam Navarro, Michel Peuchmaur), and assigned 20% of these MYCN-negative tumors to the unfavorable category. The difference in event free and overall survival between the favorable and unfavorable subset was statistically significant and underlines the importance of the INPC as a valuable tool for prognostic categorization of localized, MYCN non-amplified tumors.

In the forthcoming LNESG2 study, these results have already influenced the therapeutic design in case of relapse of the localized tumor. It has been decided that the relapse will
receive chemotherapy if the histoprognostic categorization of the primary tumor was unfavorable, while the relapse of a histologically favorable tumor will be treated with surgery alone. However, according to the new protocol, the primary tumor’s histoprognostic category will only be implied into therapeutic decisions for a relapse, if the histological slides of the primary have been reviewed by one of the members of the SIOPEN Pathology Reference Panel within six weeks after the local pathologist finished his/her report. It is mandatory that the decision about favorable or unfavorable histoprognosis of the localized tumor is based on at least two pathologists from different institutions. If the local pathologist can not agree to the conclusion made by the first reviewer, another member of the SIOPEN Pathology Reference Panel will be contacted for a third opinion.

**Summary**

Pathologists are today ascribed a key role in the diagnostic work up of pNTs:

1. After macroscopic inspection, pathologists are responsible for an adequate sampling, saving and quality control of tumor material for morphological, biological and genetic investigations.
2. The result of the pathologist’s microscopic analysis assigns a tumor to one of five morphological categories which in case of stroma-rich/-dominant tumors (except for nodular ganglioneuroblastomas) render the analysis of biological markers irrelevant.

The determination of morphological differentiation and MKI enables the pathologist to categorize the neuroblastomatous tumors and nodular ganglioneuroblastoma into favorable or unfavorable subsets which will influence the choice of therapy of a possible relapse in those patients who are enrolled into the forthcoming European protocol for localized, non-MYCN amplified peripheral neuroblastic tumors.

Finally, the great importance of obtaining biopsy material, which is adequate for histoprognostic assessment, cannot be stressed enough. Cytological specimens of tumor cells, produced from fine needle aspirates, can be used for biological analyses - provided that a pathologist has controlled their tumor cell content -, but such samples preclude any histoprognostic evaluation. The biological information of a MYCN non-amplified status, obtained from cytological samples, should not make the clinician live in the belief that this is a tumor of good prognosis. Data by Goto et al [8] and Navarro et al [19], summarized in this paper, provide clear evidence that MYCN non-amplified tumors may “hide” a considerable proportion of cases with unfavorable outcome.

**Fig. 12** Event free survival for patients grouped according to FH (favorable histology), UH (unfavorable histology) and MYCN status (modified from Goto et al., 2001).

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Intracranial ependymomas in children: correlation of selected factors and event-free survival in the group of 142 consecutive patients treated at one institution.

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Abstract

Ependymomas are among the most frequent intracranial tumors in children. Nevertheless, the identification of risk factors and the role of various treatment modalities are still far from standardization. The aim of this study was to evaluate the impact of patients’ age, tumor location, extent of surgical resection, degree of histological malignancy, type of radiotherapy (RT), and chemotherapy (CHT) on event free survival in the group of 142 consecutive patients treated at one institution. Retrospective analysis and comparisons of event-free survival (EFS) probabilities were done in subgroups of patients selected on the basis of various treatment protocols. For the entire group, age of patients, extent of surgical resection, and histological malignancy significantly affected EFS. Extent of surgical resection significantly influenced EFS in benign tumors and did not in anaplastic ependymomas. Neuroaxis RT did not improve EFS for anaplastic lesions. Treatment results were better after 1997 in anaplastic ependymomas (potential role of CHT) and finally CHT significantly improved control of small tumor residues

Key words: chemotherapy, ependymoma, prognosis, radiotherapy, surgery

Introduction

Ependymomas are among the most frequent intracranial tumors in children, accounting for approximately 10% of primary central nervous system neoplasms [35]. Treatment of patients with ependymomas includes neurosurgical resection, radiotherapy (RT), chemotherapy (CHT), and radiosurgery [16, 33]. Despite introduction of various treatment regimens, treatment outcomes are still unsatisfactory [27, 31, 36].

The aim of the study was to analyse the influence of selected factors on event-free survival (EFS) in the group of intracranial ependymomas treated at our institution.

Material and methods

In the years since 1981 thru 2003, a total number of 154 children suffering from intracranial ependymomas were treated at the Children’s Memorial Health Institute in Warsaw. Twelve patients were lost to follow-up, and the remaining group of 142 children (74 boys and 68 girls) were used for further analysis.

Retrospective analysis of the data was performed to evaluate the influence of patients’ age, tumor location, extent of surgery, tumor malignancy, type of radiotherapy (RT) and use of chemotherapy (CHT) on event-free survival (EFS), according to various treatment protocols. The group of patients was heterogenous (various treatment protocols, mainly before and after 1997). Age of patients, extent of resection, and tumor malignancy were the main factors influencing selection
of treatment options. In such circumstances the mentioned factors with potential influence on survival were not independent varieties, and multivariate analysis could not be performed. We made a 2-step statistical analysis. First, comparability of the subgroups of benign and anaplastic ependymomas was ascertained, according to age, extent of surgery, type of RT, and use of CHT. Then, we made a few separate comparisons for comparable subgroups of patients in order to analyse the potential influence of risk factors on actuarial EFS. EFS probability curves were drawn, and their stratification was studied using the log-rank test.

We did not study overall survival (OS), because recurrences of tumors as well as their treatments were not analysed.

Results

Age of the patients ranged from 5 months to 18 years and 6 months (median 5 years 0 months). In 63 patients ependymomas were located in the infratentorial space, and in 59 children supratentorial tumors were found. In 61 patients the tumors were totally, in 38 subtotally, and in 38 children partially resected. In the remaining 5 cases only biopsies were performed. In the years since 1981 thru 1999, the extent of surgical tumor removal was evaluated on the basis of surgical reports and postoperative CTs. Since 1999, the inoperable estimation as well as postoperative MRI studies have been used to determine the extent of surgical resection. All pathological specimens were reviewed and divided into two subgroups of 89 benign and 53 malignant (anaplastic) ependymomas. Anaplastic tumors matched the following criteria: marked nuclear pleomorphism, high mitotic activity, necrosis, vascular proliferation. 118 patients were treated with radiotherapy. Radiotherapy significantly changed over the described period of time, due to the development of cobalt-therapy and linac technology as well as introduction of new treatment protocols. To simplify the variety of problems concerning radiotherapy, for further analysis we defined only two main subgroups: 1. “local” radiotherapy (29 patients) and 2. neuroaxis radiotherapy (89 patients). Until 1996, irrespective of the extent of surgical resection, all patients underwent whole neuroaxis irradiation. Since1996 thru 2000, patients after total removal of benign ependymomas were treated with “local” radiotherapy. Until 2000 the “wait and see” approach was introduced after total removal of benign tumors. Partial resections of benign ependymomas were followed by chemotherapy and “local” RT. Anaplastic ependymomas after surgery were treated with adjuvant chemotherapy followed by tumor bed and ventricles irradiation, and maintenance chemotherapy. Two different treatment approaches were tailored for children under 3 years of age. Total resection of benign ependymoma was the only treatment modality. The rest of patients below 3 years of age (partial resection of benign tumor and all patients with anaplastic ependymomas) were treated with 18-months’ chemotherapy without RT. The use of the whole neuroaxis RT was reserved only for disseminated tumors. Chemotherapy was used for the treatment of 58 patients. The median follow-up period was 3 years and 2 months (ranging from 2 months to 18 years and 6 months).

In the study group there were no significant differences between patients with benign and anaplastic tumors according to age and extent of resection. The use of neuroaxis vs. local RT were close to the limit of significance (Table 1). The entire study group was devided into 3 age-dependent subgroups. There were 41 children below 3 years of age. In 75 patients, age ranged from 3 to 10 years, and 26 patients were older than 10 years. The list of subsequent comparisons of potential risk factors, outcomes and statistical analysis are shown in Table 2.

The study revealed that age of patients significantly influenced EFS. The lower the age, the worse the prognosis (Fig. 1). No differences were found concerning event-free survival probabilities for the group of 83 patients with infratentorial tumors vs. 59 patients with supratentorial ependymomas. Primary tumor location did not influence EFS. Diagnosis of malignant (anaplastic) ependymoma according to the mentioned morphologic criteria was a marker of poor prognosis in the study group (Fig. 2). Event-free survival probability for the group of 99/142 patients after total and subtotal tumor resection was better than for the group of 43/142 patients after partial resection and biopsy. Extent of surgical resection significantly influenced EFS for the entire study group. Extent of surgical removal significantly influenced EFS for benign tumors. The prognosis was better for patients after total and subtotal resection of benign ependymomas (Fig. 3). Event-free survival probability after total and subtotal resection of grade III ependymomas was slightly better than after less radical resection, but stratification of survival curves was not significant. The comparison of EFS probabilities for benign ependymomas treated with neuroaxis and “local” RT revealed that EFS was better for the subgroup after whole neuroaxis irradiation. It is rather confusing, taking into account everyday clinical practice. Before 1996, all the cases of intracranial ependymomas irrespective histological malignancy and extent of surgical resection received whole neuroaxis RT, whereas after 1996 neuroaxis irradiation was reserved for disseminated lesions only. There was no statistically significant difference between EFS probabilities for neuroaxis vs. “local” (tumor bed or tumor bed + ventricles) RT for the patients with malignant ependymomas. It was shown that introduction (1997) of the standardized treatment protocols including: surgery, adjuvant chemotherapy, tumor bed or tumor bed + ventricles radiotherapy, and finally maintenance chemotherapy significantly improved EFS probability for malignant ependymomas (Fig. 4). Statistically significant stratification of EFS curves for the patients treated with chemotherapy vs. patients treated without chemotherapy after total or subtotal tumor removals indicates that chemotherapy improved control of small tumor residues (Fig. 5).

Discussion

Identification of clinical and pathological factors influencing survival has a key role in improvement of treatment outcomes in children suffering from intracranial ependymomas. Various risk factors were proposed, and because of relatively small and heterogeneous groups of patients and different treatment protocols, the results of numerous studies differ considerably
Table 1
Comparison between ependymoma grade I - II and ependymoma grade III patients according to: age, extent of surgery, type of RT, and use of CHT (NS=not significant)

<table>
<thead>
<tr>
<th>Feature</th>
<th>Benign vs. anaplastic (ch²)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>P &gt; 0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Surgery</td>
<td>P &gt; 0.05</td>
<td>NS</td>
</tr>
<tr>
<td>RT (CNS vs. local)</td>
<td>P = 0.06</td>
<td>+/-</td>
</tr>
<tr>
<td>CHT</td>
<td>P &lt; 0.0005</td>
<td>***</td>
</tr>
</tbody>
</table>

Table 2
List of comparisons of the studied factors, and their significance (NS=not significance, *, **, *** = degrees of statistical significance)

<table>
<thead>
<tr>
<th>Comparisons of EFS probabilities for the studied parameters</th>
<th>Log-rank</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three age groups</td>
<td>p= 0.0039</td>
<td>**</td>
</tr>
<tr>
<td>Infratentorial vs. supratentorial ependymomas</td>
<td>p=0.37</td>
<td>NS</td>
</tr>
<tr>
<td>Benign vs. anaplastic ependymomas</td>
<td>p=0.0037</td>
<td>**</td>
</tr>
<tr>
<td>Total and subtotal resections vs. partial resections and biopsies (entire study group).</td>
<td>p=0.0001</td>
<td>***</td>
</tr>
<tr>
<td>Total and subtotal resections vs. partial resections and biopsies (benign ependymomas).</td>
<td>p=0.0005</td>
<td>***</td>
</tr>
<tr>
<td>Total and subtotal resections vs. partial resections and biopsies (anaplastic ependymomas).</td>
<td>p=0.15</td>
<td>NS</td>
</tr>
<tr>
<td>Neuroaxis RT vs. “local” RT (benign ependymomas)</td>
<td>p=0.007</td>
<td>**</td>
</tr>
<tr>
<td>Neuroaxis RT vs. “local” RT (anaplastic ependymomas)</td>
<td>p=0.6326</td>
<td>NS</td>
</tr>
<tr>
<td>Anaplastic ependymomas treated before 1997 vs. after 1997.</td>
<td>p=0.043</td>
<td>*</td>
</tr>
<tr>
<td>Totally and subtotally removed tumors CHT (+) vs. CHT (-)</td>
<td>p=0.047</td>
<td>*</td>
</tr>
</tbody>
</table>

[2, 6, 9, 19, 25]. Lower age of patients was found to be a poor prognostic factor in most series [3]. It was not clear whether poorer prognosis in very young children was due to different tumor biology, different treatment protocols, higher morbidity and poorer response to various treatment modalities especially radiotherapy [34]. Our results confirmed that observation. EFS probabilities were lower for the subgroups of younger children. The influence of primary tumor location on survival was difficult to evaluate reliably. Predominance of infratentorial ependymomas in the first decade of life and higher frequency of supratentorial tumors in adolescents and adults as well as precise tumor location have led to different treatment outcomes in particular subgroups of patients. No differences were found in the study group concerning two main localization groups of supra- and infratentorial ependymomas. However, it should be remembered that surgical mortality and morbidity as well as extent of surgical resection are more favorable in supratentorial extraaxial and midline posterior fossa ependymomas as compared to midline supratentorial and posterior fossa paramedian (cerebellopontine angle) tumors [4, 20]. Extent of surgical resection was the most consistent and strongest prognostic factor in almost all of series as well as in our group of patients [24, 35]. The main problem in tailoring treatment and predicting prognosis is to define reliable features in the diagnosis of malignant (anaplastic) ependymomas. Uncritical use of the morfologic criteria of histologic malignancy accepted for astrocytomas is doubtful. In our group of patients, actuarial event-free survival was significantly better for the so called benign ependymomas, but a lot of authors indicate that there is no strict correlation between tumor morphology and prognosis in ependymomas [11, 29, 30]. Much time and effort were spent to study various immunohistochemical and genetic features as well as their correlations with tumor biology, but there are still no reliable markers of malig-
Fig. 1 Statistically significant stratification of event-free survival probability curves for various age groups of patients (log-rank: \( p = 0.0039 \))

Fig. 2 Significant stratification of EFS probability curves for benign (B - 89 patients) and anaplastic (A - 53 patients) tumors (log-rank: \( p = 0.0037 \))

Fig. 3 Significantly higher EFS after total and subtotal resections (T+ST) vs. partial resections and biopsies (P+B) of benign ependymomas (log-rank: \( p = 0.00005 \))

Fig. 4 Significant improvement of treatment outcome of anaplastic ependymomas after 1997 (introduction of complex treatment protocols: surgery + adjuvant CHT + RT + maintenance CHT; log-rank: \( p = 0.043 \))

Fig. 5 Better EFS after chemotherapy (CH +) in patients in whom total or subtotal tumor resections were performed (log-rank: \( p = 0.047 \))
nancy in ependymomas [7, 21]. Opinions on adjuvant therapy differ from series to series. It is generally accepted that radiotherapy and chemotherapy can delay tumor recurrence but their influence on overall survival is still unclear [1, 5, 23, 28]. The rationale for aggressive removal of ependymomas in certain locations is debatable. Devastating late complications of neuroaxis radiotherapy, especially in the youngest age group are well known [17, 18, 32]. That is why there is a trend to eliminate radiotherapy in children under 3 years of age and after total removal of histologically benign tumors. The fields of irradiation were restricted to tumor bed or tumor bed and ventricles for partially resected benign and anaplastic lesions respectively. The neuroaxis irradiation is usually reserved for disseminated ependymomas [12, 26]. This tendency is supported by the observations that recurrences are limited to primary tumor location in almost all cases and CSF dissemination is relatively rare [13]. Patterns of failure indicate, that final treatment outcome depends mostly on the local control of disease. These statements were the reason for the concept of “second-look” surgery, whenever reoperation was possible in cases of residual disease [8]. Chemotherapy was introduced in the treatment of children under 3 years to delay or eliminate the need for radiotherapy as well as in the treatment of malignant lesions and tumor recurrences and disseminations and finally in the treatment of partially resected benign lesions [15, 37]. Variable response rates to the same treatment regimens created the need for potential identification of subpopulations sensitive or resistant to chemotherapy, development of novel drugs and intensification of treatment with subsequent bone marrow reconstruction [10, 14, 22]. As mentioned above, in our series neuroaxis irradiation correlated with better actuarial EFS for benign ependymomas. Significant change of treatment protocols over the years resulted in fairly confusing data. Before 1996, all cases were treated with neuroaxis irradiation; after 1996, RT was reserved only for disseminated ependymomas. It was clearly shown that neuroaxis RT did not improve EFS in anaplastic ependymomas in our series. An introduction of standardized treatment protocols (including chemotherapy) in 1997 improved treatment outcomes in malignant tumors. Chemotherapy also improved control of small tumor residues after surgery. Treatment response of tiny remnants seems to be an interesting clinical model for evaluation of CHT efficacy in the treatment of ependymomas.

Conclusions

1. For the entire group of patients, age, extent of surgical resection, and histological malignancy, significantly influenced EFS;
2. Extent of surgical resection significantly influenced EFS in benign tumors and did not influence EFS in anaplastic ependymomas;
3. Neuroaxis RT did not influence EFS in patients with anaplastic ependymomas;
4. Treatment results in anaplastic ependymomas improved after 1997 (a potential role of CHT);
5. CHT significantly improved control of small tumor residues,

References

Pathology of fatty liver in children with LCHAD deficiency

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Abstract

Deficiency of long chain 3-hydroksyacyl-CoA dehydrogenase (LCHADD) impairs the process of mitochondrial beta-oxidation of fatty acids. LCHADD may be clinically benign up to the moment of first symptoms, i.e. fasting inducing the first acute life-threatening episode (ALTE). Patients present vomiting, lethargy, convulsions, arrhythmia and hypoketotic hypoglycemia, which is essential for established a diagnosis. Thus, after fasting, healthy looking child may die with serious symptoms of acute illness. The aim of this study was the histopathological assessment of liver in our own LCHADD patients with particular attention to characteristic changes including intensity, type and localization of liver steatosis. Seven liver samples were assessed: 3 biopsies and 4 autopsy specimens obtained from 6 patients, aged from 3 months to one year. In 3 autopsies we found severe macrovacuolar fatty liver, in one biopsy moderate mixed steatosis, and in remaining 3 cases mild fatty liver of macrovacuolar and mixed character. All samples but one displayed diffuse distribution of steatotic hepatocytes. In 4 cases steatosis was accompanied by fibrosis and cirrhosis, in 3 by inflammatory infiltrates. Intensity of steatosis was related to the severity of clinical symptoms. Fibrosis was another morphological feature characteristic for LCHAD deficiency. The results showed that liver steatosis found during autopsy of patients who died of unexplained reason should attract attention to LCHADD as a possible cause of death.

Key words: LCHAD deficiency, liver steatosis

Introduction

Clinical symptoms of long chain 3-hydroksyacyl-CoA dehydrogenase (LCHAD) deficiency usually appear after prolonged fasting or could be evoked by upper respiratory tract infections, vaccination, physical exercises and other stress. Clinically healthy child presents hypoketotic hypoglycemia, which may be accompanied by vomiting, abdominal pain, convulsion, muscle weakness and cardiomegaly. In the course of the disease sensorimotor neuropathy and pigmentary retinopathy develop [5, 9, 11]. The age of onset ranges from one day to 40 months. Treatment involves the diet with long chain fatty acids restriction and increased supply of carbohydrates.

Fatty acids are the most important source of energy during fasting [5, 11]. They are transported into the inner membrane of mitochondria to be used in beta-oxidation process [5, 9, 11]. This process has especially important value for muscle, heart and liver cells as their own source of energy. Beta-oxidation process has two steps: transport and following oxidation. Three enzymes take parts in transport of fatty acids into the mitochondria, creating the pathway called carnitine transporting system [2, 3, 5, 12]. Proper beta-oxidation pathway depends on activity of several enzymes (four for long, four of medium and four for short chain fatty acids). The names of appropriate enzymes differ only by the name of length of the chain. The basis of beta-oxidation process is shortening of fatty acid chain by cutting acetyl-CoA to introduce it to tricarboxylic acids cycle. Deficiency of 3-hydroksyacyl-CoA dehydrogenase (LCHAD) impairs the process and leads to toxic accumulation of fatty acids and their acylcarnitines derivatives [2, 5, 7, 9, 11]. Accumulation of lipids in the cells may be a site effect of this metabolic decompensation.

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Since 1994, twenty two unrelated children with LCHAD deficiency have been identified in Poland. Diagnosis was done on the analysis of GC-MS organic acid profile in urine and than confirmed by genetic investigation. Common protocol of treatment and monitoring (CPTM) has been introduced in 2000. There were 8 LCHAD-deficient children identified before CPTM. Diagnosis was markedly delayed in all cases, rarely established at first acute life-threatening episode (ALTE), frequently post mortem. Five of them died, only three patients were in good condition. In second period (since 2000) 14 new LCHAD deficient cases were identified. The diagnosis was established usually at first ALTE, only in 4 patients too late (post mortem or post-episodic neurological sequels).

As in the literature there are only few reports concerning histological pattern of the liver from patients with LCHAD deficiency [5, 7, 9, 11], the aim of our study was to analyse morphological findings in the liver of 6 patients with LCHAD deficiency to establish the spectrum of morphological changes and compare our results with the described reports.

**Material and methods**

Material consists of seven liver samples obtained from six LCHAD deficient patients (4 boys and 2 girls). One of the patients underwent liver biopsy at the age of 3 months and post mortem examination at 6 months. There were three biopsy samples obtained from the LCHAD-deficient boys and four autopsy specimens derived from affected patients (two boys and two girls). Patients were aged from 3 to 12 months. Clinical diagnosis of LCHAD deficiency was based on urine organic acid profile analysis using GC-MS method (Dept of Laboratory Diagnostics, CMHI), and confirmed by molecular study.

The liver samples obtained during biopsies were prepared for light and electron microscopy. Slides were stained with hematoxylin and eosin, PAS, diastase digested PAS, for collagen and reticulin fibres – AZAN and Gomori stains, respectively. Semi-thin slides of plastic embedded (epon) liver samples post fixed in osmium tetroxide and stained with toluidine blue were used for steatosis detection. Liver tissue samples obtained during autopsies were processed routinely for histology and stained with hematoxylin and eosin.

During microscopical assessment we assumed three grades of steatosis intensity: mild, moderate and severe, when lipid droplets comprised up to 30%, 30-70%, and over 70% of liver parenchyma, respectively. Two types of distribution of steatotic hepatocytes were recorded: diffuse or focal. Type of steatosis included three categories: macrovacuolar, microvacuolar, and mixed. Semi-thin slides are very helpful in correct lipid detection, especially of microvacuolar type. Accompanying inflammatory infiltrates and fibrosis were assessed according to routine grading and staging system.

**Results**

**Case No 1**
The liver specimen has been obtained on autopsy. It revealed the diffused localization of mixed pattern of steatosis concerning of 100 % of hepatocytes. Apart of congestion any other pathological change was not detected in the liver. Steatosis of cardiomyocytes and lipid accumulation in renal proximal tubules was observed as well (Fig. 1).

![](image1.png)

**Case No 2**
The liver specimen has been obtained on autopsy. It revealed the typical pattern of fatty liver. Macrovesicular lipid droplets are present in almost all hepatocytes. Mild fibrosis was present only in periportal areas. Inflammatory infiltrates and cholestasis were not detected. There was found vacuolisation corresponding probably to lipid accumulation in proximal renal tubules (Fig. 2).

![](image2.png)
Liver biopsy of patient no 2 shows severe diffuse macrovesicular steatosis. Liver biopsy of patient no 3 shows diffuse, moderate, mixed steatosis. Mild intralobular inflammatory infiltrates of lymphoid cells and single necrotic hepatocytes are observed.

**Case No 3**
The liver tissue obtained during biopsy showed fatty changes in approximately 60% of hepatocytes. They revealed diffuse distribution. Lipid droplets in hepatocytes were of mixed pattern: macrovacuoles and microvacuoles were present. The mild intralobular and portal inflammatory infiltrates of lymphoid cells were found. Single necrotic hepatocytes were observed. Fibrosis and cholestasis were not detected (Fig. 2).

**Case No 4**
The liver biopsy specimen obtained prior to autopsy revealed precirrhotic pattern of liver injury. Macrovesicular steatosis were seen in 30% of hepatocytes. Their localization was rather focal, within some of the nodules. Severe portal inflammatory infiltrates were present. Cholestasis was not found (Fig. 3).

**Case No 5 (the same patient as above)**
Subsequent liver specimen obtained during autopsy revealed cirrhosis. Severe macrovesicular fatty changes concerned all hepatocytes. Mild portal inflammatory infiltrates remained similar to those of the biopsy specimen. No cholestasis was present (Fig. 3).

**Case No 6**
The liver biopsy differed substantially from other patients. Diffuse, mixed steatotic changes were seen only in about 5-10% of hepatocytes. No inflammatory infiltrates, fibrosis or cholestasis were detected in the liver.

**Case No 7**
The girl was referred to autopsy examination with diagnosis of cardiac insufficiency due to hypertrophied cardiomyopathy. Appropriate diagnosis was established retrospectively, some years after death on the basis on genetic family study. It was performed after her younger brother was diagnosed to have LCHAD deficiency. On autopsy examination features of excentric hypertrophy of left cardiac ventricle with accompanying signs of mitral insufficiency and endocardial thickening was observed. Steatosis of the liver was also reported in autopsy protocol, but no morphological characteristic was made. Microscopically, macrovesicular steatosis of diffuse localization consisted of 10% of hepatocytes accompanied by mild fibrosis was found. No inflammation and cholestasis was observed.
Summary

Liver steatosis of severe degree involving 100% of liver parenchyma was found in three patients (No 1, 2, and 5) who died during ALTE. Moderate steatosis involving approximately 60% of liver was present in one biopsy (No 3). Mild steatosis affecting 30% and 15% of liver parenchyma was found in two biopsies coming from cases 4 and 6, respectively. In one autopsy sample liver steatosis involved 10% of parenchyma (No 7). Steatosis was macrovacuolar or mixed. The first type was found in two sequential examinations of the same patient (biopsy – No 4 and autopsy – No 5) and in two autopsies (samples No 2 and 7). Macrovacuolar steatosis was severe in two cases (No 2 and 5) and mild in two others (No 4 and 7). Mixed steatosis was seen in two biopsies (No 3 and 6) and one autopsy (No 1). Its degree was mild (sample No 6), moderate (sample No 3), and severe (sample No 1). None of the patients had pure microvacuolar fatty liver. In six cases distribution of steatotic hepatocytes was diffuse, in one focal (sample No 4). Inflammation was present in three cases. It was mild (cases No 3 and 5) or severe (case No 4). Fibrosis was disclosed in four examined samples derived from three patients: in two cases its intensity was mild (No 2 and 7) in two severe (samples No 4 and 5). Liver biopsy samples available for assessment from the same patient disclosed progression from precirrhosis to cirrhosis. Data concerning patients and their liver morphology are presenting in Table 1.

Discussion

It is known that the process of mitochondrial fatty acid oxidation can be divided in two stages. First involves transport through mitochondrial membrane, the second consists of cutting off two-carbon moieties from fatty acid chain by several enzymes for long, medium, and short chain fatty acids, respectively [5, 9, 11]. Fatty liver may develop in both patients with defects of the first stage involving carnitine dependent transport [2, 3, 12], and with defects of the proper beta-oxidation [2, 4, 6-8, 10, 11]. Baldridge in his study of 82 patients with liver steatosis reported only one child in whom the LCHAD deficiency was established [1]. Our group comprises six patients with this diagnosis. Roe and Coates provided general description of liver morphology in LCHAD deficiency [9]. They pointed lipid accumulation and sometimes accompanying fibrosis as characteristic features of this disorder. Our findings are consistent with this observation. However, they have not provided particulars concerning intensity, type, and distribution of steatosis. Gilbert-Barness and Barness have presented more detailed description of liver microscopical pathology in LCHAD deficient patients [5]. They considered diffuse or zonal macrovacuolar severe fatty liver to be characteristic of the majority of LCHAD-deficient patients. In our group this type of morphological pattern was found in three patients always when the liver biopsy was performed at the acute clinical presentation.

In a number of described patients with LCHAD deficiency the liver discloses only focal steatosis of mild intensity [5]. We observed focal fatty changes only in one case out of seven (sample No 4). In remaining patients the degree of steatosis was mild to moderate and was almost invariably diffusely distributed.

Moore described morphology of the liver in six months old girl with LCHAD deficiency [7]. There were mixed steatosis accompanied by mild cholestasis. Author has not provided data concerning intensity and localization of fatty changes. In our series of seven cases we did not find any case with the features of cholestasis.

Tyni described liver morphology including microscopical findings of 16 patients coming from 12 families, who died with diagnosis of LCHAD deficiency [11]. All patients in her study had features of macrovacuolar fatty liver. In our patients steatosis was not only macrovacuolar, but also mixed. Tyni concluded that steatosis was typical for fatty acid beta oxidation defects, and emphasized that accompanying fibrosis was

Table 1

Details of morphological changes of the liver in patients with LCHADD

<table>
<thead>
<tr>
<th>Type of examination</th>
<th>Sample No</th>
<th>Age (mo)</th>
<th>Intensity of steatosis (%)</th>
<th>Type of steatosis</th>
<th>Localization of steatosis</th>
<th>Inflammation</th>
<th>Fibrosis</th>
<th>Cholestasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3299</td>
<td>4</td>
<td>100</td>
<td>mixed</td>
<td>diffuse</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>A</td>
<td>3289</td>
<td>3</td>
<td>100</td>
<td>macro</td>
<td>diffuse</td>
<td>no</td>
<td>mild</td>
<td>no</td>
</tr>
<tr>
<td>B</td>
<td>30573</td>
<td>12</td>
<td>60</td>
<td>mixed</td>
<td>diffuse</td>
<td>mild</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>B</td>
<td>30760</td>
<td>3</td>
<td>30</td>
<td>macro</td>
<td>focal</td>
<td>severe</td>
<td>precirrhosis</td>
<td>no</td>
</tr>
<tr>
<td>A</td>
<td>2701</td>
<td>6</td>
<td>100</td>
<td>macro</td>
<td>diffuse</td>
<td>mild</td>
<td>cirrhosis</td>
<td>no</td>
</tr>
<tr>
<td>B</td>
<td>51605</td>
<td>7</td>
<td>15</td>
<td>mixed</td>
<td>diffuse</td>
<td>no</td>
<td>no</td>
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</tr>
<tr>
<td>A</td>
<td>3070</td>
<td>6</td>
<td>10</td>
<td>macro</td>
<td>diffuse</td>
<td>no</td>
<td>mild</td>
<td>no</td>
</tr>
</tbody>
</table>
especially typical for LCHAD deficiency [11]. In our series the fibrosis was found in four out of seven liver samples (obtained from three children). Tyni suggested presence of relationship between the severity of clinical course and the intensity of morphological changes in the liver [11]. Our observations are in agreement with this statement.

Our observations strongly underline that liver steatosis of any degree detected at autopsy of a child died without clearly established diagnosis obliges to include fatty acids oxidation disorders into the differential diagnosis [2]. One of our patient, died many years ago with the diagnosis of hypertrophied cardiomyopathy, was detected just recently as LCHAD-deficient during genetic family study, after establishing LCHAD deficiency in her younger brother. Lack of acute life threatening episode in her medical history and only mild degree of liver steatosis in her post mortem examination, preliminarily did not paid our attention and missed from correct diagnosis.

In general our results are consistent with observations of other authors and warrant drawing the following conclusions:
1. Fatty liver is characteristic of LCHAD deficiency.
2. Intensity of steatosis is related to severity of clinical symptoms.
3. In patients with mitochondrial beta-oxidation defects liver steatosis may be accompanied by fibrosis which is suggestive of LCHAD deficiency.
4. Fatty liver found in a patient who died of undiagnosed disease should suggest the possibility of LCHAD deficiency as a cause of death.
5. Semi-thin plastic embedded tissue section postfixed in osmium tetroxide and stained with toluidine blue is very helpful in reliable assessment of liver steatosis.

Acknowledgments
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References
Isolation of urothelial cells for tissue engineered bladder augmentation

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Abstract

Objectives. In case of certain human congenital anomalies such as bladder extrophies, there may not be enough residual bladder for closure. Tissue engineering is a promising method for bladder augmentation. The aim of our study was to compare isolation efficiency of various digestive mixtures and establish isolation and cell culture methodology of urothelium. Different variants of growth media and their influence on proliferative capacity was assessed. Methods. Primary Rabbit Urothelial Cells (PRUC) culture was established from 8 months old male rabbit urinary bladder wall. 5 different digesting mixtures for isolation of urothelial cells were tested. Cells obtained from 0.1% collagenase I digestion after 1 and 4 hours were checked for their growth potential in 3 different media. Results. The best digestion solution for urothelial cells isolation was 0.1% collagenase I. It can be noticed that the cell number of all secondary cultures was higher after 4 h digestion than after 1 h. The medium substrates also influenced total cell number in urothelial subcultures. The best foetal bovine serum (FBS) supplementation was 5%, when the medium was additionally enriched with micro-supplements. Conclusions. Despite of enzymes used for isolation, the digestion time and medium enriched with properly amount of serum both deeply influence on the outcome of cell therapy. Autologous cells could be used for urinary tract reconstruction using tissue engineering methods.

Key words: tissue engineering, urinary tract reconstruction, urothelial cells

Introduction

In case of certain human congenital anomalies such as bladder extrophies, there may not be enough residual bladder for closure [7]. The autologous materials and techniques have been proposed for bladder augmentation with small and large bowel, stomach [1, 13]. Unfortunately none of used materials has been enough satisfactory [9, 17]. A number of biomaterials have been used in experimental models including acellular tissue matrix grafts, synthetic biodegradable or non-biodegradable polymers [2]. Many reports revealed failure of these attempts because of recurrent urinary tract infections, marked contracture of the graft or graft rejection and stones formation [5, 12, 15, 17]. Tissue engineering is a promising method for bladder augmentation [7]. The use of an autologous cultured urothelium for bladder reconstruction could prevent many of the problems associated with applied materials [4]. Tissue engineered bladder wall consisting of a adequate shaped biodegradable polymer used as a scaffold, can deliver autologous urothelial cells [11]. This device may restore bladder capacity more effective than biomaterials alone. Atala et al demonstrated that urothelial cells seeding on the bioabsorbable scaffold can allow creation of new functional urinary bladder wall [3].
The aim of our study was to compare isolation efficiency of various digestive mixtures and establish proper isolation methodology of urothelial cells. Also different variants of growth medium and its influence on proliferative capacity of growing cells was assessed. In the future these cells can be used for urinary tract reconstruction using tissue engineering methods.

**Methods**

Primary Rabbit Urothelial Cells (PRUC) culture was established from 8 months old male rabbit urinary bladder wall. After ketamine (25 mg/kg m.c. i.m.) and scoline (50 mg/kg) overdosage bladder wall sample (5 x 5 cm) was obtained. Fragment was immersed in sterile Phosphate Buffered Saline (PBS, Biomed). In the next step urinary mucosa was mechanically separated from muscle layer. At this stage samples were cut into 12 equal sized parts (1 cm² each). Mucosa samples were cut into 2 mm³ pieces.

First of all 5 different digesting mixtures for isolation of urothelial cells were tested. Incubation time was 4 hours. Digesting solutions were as follow:

1. 0.125% trypsin and 0.01% EDTA
2. 0.08% trypsin and 0.006% EDTA
3. 0.06% trypsin and 0.005% EDTA
4. 0.05% trypsin and 0.02% EDTA
5. 0.1% collagenase-I

After incubation time the cell pellet was centrifuged (800 g/5 min), resuspended in each of the tested culture media and seeded on 25 cm² culture dishes (Greiner). Cells were identified as epithelial cells by morphological criteria as well as presence of cytokeratins (Anti-Cytokeratin, Clone: MNF 116, DAKO), after three passages.

In the next step 2nd PRUC was established using the most efficient digestive solution: 0.1% collagenase I. Digestion efficiency was assessed using two incubation times (1 and 4 hours). Cells obtained from 0.1% collagenase I digestion after 1 and 4 hours were checked for their growth potential in 3 different media:

- I- DMEM supplemented with 10% FBS and antibiotics: penicillin (100 IU/ml) (Polfa) and streptomycin (100 µg/ml) (Polfa)
- II- 3:1 DMEM and F-12 mixture supplemented with 5% FBS, epidermal growth factor (EGF, Sigma), bovine pituitary extract (Sigma), cholera toxin (30 ng/ml) (Sigma), penicillin (100 IU/ml) (Polfa) and streptomycin (100 µg/ml) (Polfa)
- III- 1:1 DMEM and F-12 mixture supplemented with 2.5% FBS, epidermal growth factor (EGF, Sigma), bovine pituitary extract (Sigma), cholera toxin (30 ng/ml) (Sigma), penicillin (100 IU/ml) (Polfa) and streptomycin (100 µg/ml) (Polfa).

Cells were seeded on 25 cm² culture dishes (Greiner) and grown in 37°C in a humidified atmosphere with 5% CO₂. Cells were counted using trypan blue exclusion test after four days of cultivation. Neubauer cytologic chamber was used for counting. Morphology was examined under inverted microscope. Photographic documentation was done.

Mean values (+/-SD) from 10 counts for each culture were compared to each other. The Student test was used for statistical analysis. A p value <0.05 was considered as statistically significant.

**Results**

The best digestion solution for urothelial cells isolation was 0.1% collagenase-I. There were 3.17 x 10⁶ cells in 96h culture after 0.1% collagenase digestion (p<0.001 when compared to all trypsin solutions). 0.05% trypsin and 0.02% EDTA digestion solution isolated 0.57 x 10⁶ cells from rabbit bladder wall. None of the other trypsin concentrations was superior in the term of cell isolation (Fig.1).

Isolation time influenced on total cell number in primary culture. Specimens incubated for one hour in 0.1% collagenase gave 1.45 x 10⁶ cells in culture. The same collagenase concentration gave 1.71 x 10⁶ cells after 4 hours of digestion. The difference between cell number in two incubation times was statistically significant (p<0.05) (Fig.2). It can be noticed that the cell number of all secondary cultures was higher in 4 hours digestion group than in 1 hour (Fig.3).

The medium substrates also influenced total cell number in urothelial subcultures. The best foetal bovine serum (FBS) supplementation was 5%, when the medium was additionally enriched with micro-supplements. Ten and 2.5% of FBS with supplementation resulted in the similar and lower cell number after 96th of subculture (Fig.3).

**Discussion**

Cell culture is an important technology in biomaterial research and tissue engineering. Tissue engineering of urothelial organs is of interest in children, because the number of complications and re-operations may be reduced [10].

Urothelium was reproducibly cultured from all isolations (Fig.1). Cell growth could be induced onto collagen or polylactic acid matrices and other shaped surfaces. Cells from all isolations formed confluent layers of flat cells, resembling...
Digestion efficiency using 0.1% collagenase I solution was assessed using two incubation times (1 and 4 hours).

Fig. 2 Cells obtained from 0.1% collagenase I digestion after 1 hour (a,b,c) and 4 hours (d,e,f) were checked for their growth potential in 3 different media as: - DMEM with 10% FBS (a,d); - 3:1 DMEM and F-12 mixture enriched with microsupplements and 5% FBS (b,e); - 1:1 DMEM and F-12 mixture enriched with microsupplements and 2.5% FBS (c,f). Cells were counted after 4 days of culturing (A vs D, p<0.05; B vs C and E vs F, p<0.05).

Fig. 3 Cells obtained from 0.1% collagenase I digestion after 1 hour (a,b,c) and 4 hours (d,e,f) were checked for their growth potential in 3 different media as: - DMEM with 10% FBS (a,d); - 3:1 DMEM and F-12 mixture enriched with microsupplements and 5% FBS (b,e); - 1:1 DMEM and F-12 mixture enriched with microsupplements and 2.5% FBS (c,f). Cells were counted after 4 days of culturing (A vs D, p<0.05; B vs C and E vs F, p<0.05).

Fig. 4 Urothelial monolayer obtained after 4 hour digestion with 0.1% collagenase I and cultured in DMEM supplemented with 10% FBS and antibiotics (reversed microscope, magnification 100x)
References


Status of oral hygiene and dentition in children exposed to anticancer treatment

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Abstract

**Background:** Deleterious effects of radiation and chemotherapy on oral cavity tissues are well known, but little data exist on the effect of combined anticancer treatment on developing teeth. Additionally, there are no established standards for dental care in children undergoing complex anticancer treatment. **The aim of study:** Evaluation of oral hygiene and dentition status in children subjected to combined anticancer treatment. **Material and method:** 88 children (age range 3-18 years), who underwent combined chemo-radiotherapy or chemotherapy alone due to a malignant neoplasm, were included to the study. Follow-up time was from 6 months to 5 years. Patients were assessed in the dental out-patients' clinic and their medical documentation reviewed. **Results and conclusions:** An inadequate oral hygiene was found in all patients as well as severe caries and an increased incidence of non-caries-mediated enamel defects. A deleterious effect of combined chemo-radiotherapy on mineralized tooth tissues was found, both in primary and in permanent teeth. Ionizing radiation had a stronger negative impact than chemotherapy alone. In all children analyzed, there was a discrepancy between actual requirements for dental care and currently performed dental treatment.

**Key words:** children, chemotherapy, enamel structure, oral hygiene, permanent dentition, primary dentition, radiotherapy

Introduction

Anticancer treatment, including chemotherapy and radiotherapy of the facial region, may enhance both the development of dental caries and also of non-caries dependant lesions on smooth surfaces of teeth [4-6, 10, 11]. The etiology of these phenomena is multi-factorial and still poorly understood. Both chemo- and radiotherapy increase the susceptibility of teeth to noxious factors and affect adversely the ecosystem of oral cavity [9, 10]. In patients undergoing combined anticancer treatment, these adverse effects may enhance each other [7].

In general opinion, the direct effect of ionizing radiation on teeth leads to denaturation of protein matrix of the dentin and to damage of its structure due to degeneration and dysfunction of dental pulp. On the other hand, there is much less information concerning the pathogenesis of enamel lesions in

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available literature. Enamel is described as poorly mineralized, less hard and more brittle, or as more susceptible to damage by acids while preserving normal hardness. Independent on the mechanism of radiation-induced lesions in mineralized tissues of teeth, they become more susceptible to harmful factors [5]. There is little data concerning the effects of chemotherapy not combined with radiotherapy on teeth. Nevertheless, they show its deleterious effect on the function of dental pulp, thereby increasing susceptibility of teeth to various pathologic processes [10]. The effect of particular cytostatics on tooth pulp is difficult to ascertain. This is due to general use of multi-drug chemotherapy regimens. Toxic effects of vincristin are known the best. Histopathologic examination of teeth extracted from patients undergoing multi-drug chemotherapy revealed linear lesions, most probably resulting from disturbed production of collagen matrix by odontoblasts. An important point is finding of correlation between the number and distribution of these lines and chemotherapy cycles, when one of administered drugs was vincristin [6]. It is also well known that colchicin and vinblastin block dentin formation in incisors in rats, while trethanomelamin and cyclophosphamide delay cutting of molars in these animals [6, 7].

Adverse change of oral eco-system in patients undergoing anticancer treatment is caused mainly by qualitative and quantitative disturbances of salivary secretion [9]. Facial irradiation often leads to dysfunction of serous cells of parotid gland. Depending on total absorbed dose of radiation (over 60 Gy), this may lead to total (irreversible) or partial (reversible) xerostomia and to qualitative change of saliva, such as increased viscosity, decreased buffering properties and decreased IgA level. A decreased volume of secreted saliva may accompany administration of many cytostatic drugs [2, 4, 9]. Increased acidity of oral cavity may be further enhanced by vomiting, which often accompanies chemotherapy. An extremely unfavorable aspect of chemotherapy is its prolonged administration, ranging from 6 months to 3 years, depending on the kind of neoplasm.

Unfavorable alteration of oral eco-system in patients undergoing oncologic treatment may enhance demineralization of teeth, thus increasing the susceptibility of teeth to caries-promoting bacteria, leading to the development of caries and erosion of enamel. Particular form of pathologic process (caries and/or erosion) depends mainly on acid pH and duration of its contact with mineralized tissues of teeth [2]. Increased susceptibility of teeth to the influence of deleterious factors (chemical, bacterial and mechanical) may be an important factor particularly in children and adolescents, where it is caused not only by the neoplasm itself and anti-cancer therapy, but also by morphologic and functional immaturity of teeth [15, 16].

When discussing the etiology of damage to mineralized tissues of teeth observed in patients undergoing anti-cancer treatment, we must also take into account the effect of neurologic deficits, e.g. weakness of chewing muscles leading to “lazy” chewing and compromising self-purification of oral cavity. Other important factors are: co-existing stomatitis, disturbed taste, lack of appetite and general malaise, leading to hygienic neglect and dietary mistakes. There is no published data concerning the profile of structural changes in mineralized tissues of teeth, developing in children undergoing chemotherapy combined with facial irradiation.

**Aim of the study**

The aim of this study was clinical assessment of lesions in mineralized tissues of teeth in children subjected to chemotherapy combined with radiotherapy to the facial region and an electron-microscopic analysis of structure of atypical defects present on smooth surface of primary teeth.

**Material and method**

**Patients.** The study included 88 children (57 boys and 31 girls) aged from 3 to 18 years, who underwent combined anti-cancer treatment. Time since completion of oncologic treatment ranged from 6 months to 5 years. Patients’ characteristics concerning basic diagnosis, total dose of radiation and location of irradiated area (face or neurocranium), are presented in Table 1. Children included in the study have been subdivided into 3 subgroups: those with primary dentition, those with mixed dentition and those with permanent dentition (Table 2).

**Stomatologic assessment of dentition.** Dental examinations were performed in the setting of a dental office, using a shadowless lamp, dental probe and dental mirror. Primary assessed parameters included: oral hygiene and dental status, presence of caries, fillings, non-caries-mediated enamel defects and missing teeth. At the same time, prophylactic and therapeutic requirements were defined and secondary endpoints were assessed, such as frequency and severity of caries, treatment index and incidence of atypical non-caries-mediated defects.

**Oral hygiene.** Oral hygiene was assessed using the “Oral Hygiene Index” according to Green and Vermillion (OHI-S). Dental plaque stained with eosin was evaluated on buccal/labial and palatal/lingual surfaces of 6 representative teeth: 55 (15), 53 (13), 51 (11), 75 (36), 73 (13), 71 (31). If the desired tooth was missing, the adjacent tooth was examined. On the basis of mean values of the OHI-S index, oral hygiene was considered good if the score was 0-1.0; adequate if the score was >1.0-2.0 and poor if the score was >2.0-3.0.

**Severity of caries.** Evaluation was performed according to the DMF index for permanent teeth and the dmf index for primary teeth. The following lesions of mineralized tooth tissues were considered as atypical: white smooth stains, white or rusty rough stains, enamel defect within a white or a rusty stain.

**Structure of defect surface.** Teeth were cut along the longitudinal axis and transversely at the defect level. They were fixed in 4% glutaraldehyde solution (Serva) in 0.1M cacodylate buffer with pH value of 7.3 (BDH Chemicals Ltd, Poole, England) and next they were dehydrated using increasing concentrations of ethyl alcohol and acetone. Tooth fragments were attached to brass rods with silver paste (AGAR, England) and vacuum-coated with charcoal and gold powder and finally analyzed using a scanning electron microscope (JSM 35C, Jeol).
Table 1
Diagnosis, mean total dose and field of radiation in patients subjected to combined chemo-radiotherapy. Radiation fields included teeth and teeth buds.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of children (N)</th>
<th>Mean dose of radiation (cGy)</th>
<th>Irradiated field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medulloblastoma</td>
<td>18</td>
<td>3500</td>
<td>Neural axis (brain and spinal cord)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4500</td>
<td>Posterior fossa</td>
</tr>
<tr>
<td>Brainstem tumors</td>
<td>7</td>
<td>5400</td>
<td>Tumor site</td>
</tr>
<tr>
<td>Ependymoma</td>
<td>5</td>
<td>5400</td>
<td>Tumor site</td>
</tr>
<tr>
<td>Astrocytoma III</td>
<td>5</td>
<td>5400</td>
<td>Tumor site</td>
</tr>
<tr>
<td>Glioblastoma multiforme</td>
<td>2</td>
<td>5400</td>
<td>Tumor site</td>
</tr>
<tr>
<td>Carcinoma plexus chorioidei</td>
<td>3</td>
<td>5400</td>
<td>Tumor site</td>
</tr>
<tr>
<td>Nasopharyngeal rhabdomyosarcoma</td>
<td>9</td>
<td>5400</td>
<td>Tumor site</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>1</td>
<td>2400</td>
<td>Neural axis</td>
</tr>
<tr>
<td>Neuroblastoma stage IV</td>
<td>1</td>
<td>4500</td>
<td>Brain metastases</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>1</td>
<td>4500</td>
<td>Lymph node metastases in head and</td>
</tr>
</tbody>
</table>

In the area of dental therapeutic requirements (Table 6), the need for conservative treatment significantly outnumbered all other parameters. On the basis of proportion of teeth with active caries, need for conservative treatment was observed in 71.42 % of teeth in the subgroup of children with primary dentition, in 48.82 % of primary teeth and 89.10 % of permanent teeth in the subgroup with mixed dentition and in 95.22 % of teeth in the subgroup of children with permanent dentition.

An analysis of severity of caries is presented in Table 4. The mean dmf score was 14.6, 7.06 and 6.0 in subgroups of children with primary, mixed and permanent teeth respectively, indicating extremely severe caries both in primary and in permanent dentition. In all subgroups, the proportion of primary and permanent teeth with active caries were a significant component of the mean score of dmf and DMF indices.

An analysis of treatment indices, both for primary and for permanent teeth, showed significant neglect concerning both treatment and prevention (Table 5). Obtained values were very small, ranging from 0.02 to 0.43, depending on sex and type of dentition.

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Results

Clinical evaluation
The incidence of caries in the studied population of children after combined chemo-radiotherapy was high and approached 100 %. Oral hygiene in the whole group studied was considered adequate (mean OHIS score = 1.88), while in the subgroup of children with primary teeth oral hygiene was poor (mean OHIS score = 2.25) (Table 3).

An analysis of severity of caries is presented in Table 4. The mean dmf score was 14.6, 7.06 and 6.0 in subgroups of children with primary, mixed and permanent teeth respectively, indicating extremely severe caries both in primary and in permanent dentition. In all subgroups, the proportion of primary and permanent teeth with active caries were a significant component of the mean score of dmf and DMF indices.

An analysis of treatment indices, both for primary and for permanent teeth, showed significant neglect concerning both treatment and prevention (Table 5). Obtained values were very small, ranging from 0.02 to 0.43, depending on sex and type of dentition.
### Table 3

Correlation of oral hygiene with age and sex in children subjected to chemio- and radiotherapy (OHI-S mean value)

<table>
<thead>
<tr>
<th>Dentition type</th>
<th>Sex</th>
<th>N (children)</th>
<th>OHI-S (mean value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary dentition</td>
<td>Boys</td>
<td>7</td>
<td>2.51</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>3</td>
<td>1.63</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>10</td>
<td>2.25</td>
</tr>
<tr>
<td>Mixed dentition</td>
<td>Boys</td>
<td>19</td>
<td>1.90</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>11</td>
<td>2.01</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>30</td>
<td>1.94</td>
</tr>
<tr>
<td>Permanent dentition</td>
<td>Boys</td>
<td>17</td>
<td>1.70</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>8</td>
<td>1.55</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>25</td>
<td>1.65</td>
</tr>
<tr>
<td>Total</td>
<td>Boys</td>
<td>43</td>
<td>1.92</td>
</tr>
<tr>
<td></td>
<td>Girls</td>
<td>22</td>
<td>1.79</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>65</td>
<td>1.88</td>
</tr>
</tbody>
</table>

### Table 4

Correlation of Severity of caries, sex and type of dentition in children subjected to chemotherapy combined with radiotherapy (mean scores of dmf and DMF indices)

<table>
<thead>
<tr>
<th>Dentition type</th>
<th>Sex</th>
<th>N (children)</th>
<th>with caries</th>
<th>removed</th>
<th>filled</th>
<th>dmf</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary dentition</td>
<td>boys</td>
<td>7</td>
<td>105</td>
<td>-</td>
<td>0</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>girls</td>
<td>3</td>
<td>28</td>
<td>-</td>
<td>0</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>10</td>
<td>133</td>
<td>-</td>
<td>0</td>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td>Mixed dentition</td>
<td>boys</td>
<td>19</td>
<td>104</td>
<td>102</td>
<td>13</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>girls</td>
<td>11</td>
<td>66</td>
<td>54</td>
<td>2</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>30</td>
<td>170</td>
<td>156</td>
<td>15</td>
<td>27</td>
<td>24</td>
</tr>
<tr>
<td>Permanent dentition</td>
<td>boys</td>
<td>17</td>
<td>-</td>
<td>191</td>
<td>-</td>
<td>1</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>girls</td>
<td>8</td>
<td>-</td>
<td>81</td>
<td>-</td>
<td>3</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>25</td>
<td>-</td>
<td>272</td>
<td>-</td>
<td>4</td>
<td>168</td>
</tr>
</tbody>
</table>

- **d/D** - caries in primary/permanent dentition
- **m/M** - removed primary teeth/permanent
- **f/F** - filled primary teeth/permanent
- **du/DF** - dental caries severity index in primary/permanent dentition
### Table 5
Correlation of teeth-treatment index, dentition type and sex in children subjected to combined chemo-radiotherapy

<table>
<thead>
<tr>
<th>Dentition type</th>
<th>Sex</th>
<th>N (children)</th>
<th>Teeth-treatment index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>boys</td>
<td>7</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>girls</td>
<td>3</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>10</td>
<td>0.09</td>
</tr>
<tr>
<td>Mixed</td>
<td>boys</td>
<td>19</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>girls</td>
<td>11</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>30</td>
<td>0.14</td>
</tr>
<tr>
<td>Permanent</td>
<td>boys</td>
<td>17</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>girls</td>
<td>8</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>25</td>
<td>0.38</td>
</tr>
</tbody>
</table>

### Table 6
Correlation of requirement for conservative and surgical treatment with sex and dentition type in children subjected to chemo-therapy combined with radiotherapy

<table>
<thead>
<tr>
<th>Dentition type</th>
<th>Sex</th>
<th>N (children)</th>
<th>N (teeth)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>examined with caries n=100%</td>
<td>For conservative treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M S M S M S M S M S M S</td>
<td>n %</td>
</tr>
<tr>
<td>Primary</td>
<td>boys</td>
<td>7</td>
<td>140 - 105 - 71 67,6 - - 34 32,4 - -</td>
</tr>
<tr>
<td></td>
<td>girls</td>
<td>3</td>
<td>60 - 28 - 24 85,7 - - 4 14,28 - -</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>10</td>
<td>200 - 133 - 95 71,42 - - 38 28,57 - -</td>
</tr>
<tr>
<td>Mixed</td>
<td>boys</td>
<td>19</td>
<td>163 271 104 102 53 50,96 94 92,15 51 49,03 8 7,84</td>
</tr>
<tr>
<td></td>
<td>girls</td>
<td>11</td>
<td>102 155 66 54 30 45,45 45 83,33 36 54,54 9 16,66</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>30</td>
<td>265 426 170 156 83 48,82 139 89,10 87 51,17 17 10,89</td>
</tr>
<tr>
<td>Permanent</td>
<td>boys</td>
<td>17</td>
<td>- 451 - 191 - - 180 94,24 - - 11 5,75</td>
</tr>
<tr>
<td></td>
<td>girls</td>
<td>8</td>
<td>- 221 - 81 - - 79 97,53 - - 2 2,46</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>25</td>
<td>- 672 - 272 - - 259 95,22 - - 13 4,77</td>
</tr>
</tbody>
</table>

M- primary teeth
S- permanent teeth
Table 7

Total mean number of teeth with caries and total mean number of teeth requiring conservative and surgical treatment depending on dentition type

| Dentition type | N (teeth) (mean value) |  |
|----------------|------------------------|  |
|                | Examined               |  |
|                | M          | S     | M     | S     | M     | S     | M     | S     |
| Primary        | 10         | 20    | -     | 13,30 | -     | 9,50  | -     | 3,80  |
| Mixed          | 30         | 8,83  | 14,20 | 5,66  | 5,20  | 2,76  | 4,63  | 2,90  |
| Permanent      | 25         | -     | 26,88 | -     | 10,88 | -     | 10,36 | -     | 0,52  |

Table 8

Non-caries-dependent enamel defects in children subjected to chemo- and radiotherapy dependent on dentition type (w-labial/buccal surface, sz-cervical area)

<table>
<thead>
<tr>
<th>White smooth stain</th>
<th>Rough stain</th>
<th>Enamel defects within</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nt</td>
<td>Nt</td>
</tr>
<tr>
<td>Dentition type</td>
<td>Nc</td>
<td>Nc</td>
</tr>
<tr>
<td></td>
<td>w sz &gt;1 total</td>
<td>w sz &gt;1 total</td>
</tr>
<tr>
<td>Primary</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Mixed</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td>Permanent</td>
<td>19</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>39</td>
</tr>
</tbody>
</table>

Nc = number of children
Nt = number of teeth

Fig. 1 Scanning electron microscopic view of tooth surface with cracked enamel and altered enamel prisms within an area of enamel defect. Microscopical magnification x 1100

Fig. 2 Scanning electron microscopic view of the tooth dentin canals. Microscopical magnification x 1300
Electron-microscopic examination

Scanning electron microscopical analysis of the enamel of teeth showed its lack or crack in number of areas. Derangement of enamel prisms and their clump was observed (Fig.1). No alterations of the pattern of dentin canals were noticed (Fig.2).

Discussion

Intensification of chemotherapy and its combination with surgery and/or radiotherapy have made possible an increasingly effective treatment of head and neck malignancies [1, 2]. However, such an aggressive treatment is associated with an increased risk of many adverse side-effects, concerning many different components of the chewing apparatus. Particularly severe complications may accompany anticancer treatment of children and adolescents. Anticancer treatment proves effective in a growing number of patients, resulting in longer survival time. This in turn contributes to an increased incidence of late adverse effects of therapy, e.g. destruction of mineralized tissues of teeth and various developmental abnormalities of chewing apparatus [3, 4, 5]. Therefore, oral care professionals will be increasingly often confronted by such problems. Unfortunately, available literature provides little data concerning dental problems in patients undergoing anticancer treatment in childhood or guidelines for their effective prophylactic, therapeutic and rehabilitative management.

Results of clinical trials indicate significant hygienic neglect and poor dentition status of children subjected to chemotherapy combined with radiotherapy. We noticed alarmingly high incidence of caries and its extreme severity. In our study, the mean dmf score was 14.6, largely exceeding values observed in children in the risk group for infectious endocarditis, where dmf score was 9.25 and DMF score was 10.42, as well as in children undergoing chemotherapy alone, where it equaled to 2.5 and 14.1 respectively [13]. We have noticed a large disproportion between requirements for dental treatment and dental treatment actually carried out. Values of treatment indices in children after anticancer treatment and those in the risk group for infectious endocarditis were similar, in the range of 0-0.34 and 0.03-0.34 respectively.

An analysis of the scope of required dental treatment in children after chemotherapy combined with radiotherapy revealed significant needs in the area of conservative treatment and relatively smaller needs in the field of surgical treatment, possibly indicating high speed of development of new caries-dependent defects.

In 69% of children analyzed we observed atypical non-caries dependent enamel lesions in the form of patches and enamel defects. They occurred twice as often as in children undergoing chemotherapy alone. Children after chemotherapy combined with radiotherapy presented mostly white, rusty or rough patches, while children after chemotherapy alone had usually white lesions.

Deleterious effect of radiotherapy on dentition is well known since a long time. The so-called “post-radiation caries” in patients undergoing radiotherapy in the area of head and neck, is a phenomenon well established in the literature [2, 3, 5, 8, 11, 12, 14]. Observed thereby enamel defects are at the beginning probably non-bacterial in nature and are caused by chemical and mechanical factors acting upon a primarily more susceptible enamel. It would seem, that subsequent development of caries in these patients is secondary to the above described phenomena.

Conclusions

1. High incidence and severity of caries and atypical non-caries mediated enamel defects observed in patients subjected to chemotherapy combined with radiotherapy in the facial region, support the thesis about deleterious effect of anti-cancer on dentition.

2. Significant disproportion between requirements for dental treatment and actually performed conservative and surgical treatment indicate, that dental prophylactic and therapeutic management should be instituted as early as possible in these patients.

References


Preliminary review of the treatment results of hepatoblastoma and hepatocarcinoma in children in Poland in the period 1998-2002

Czesław Stoba 1, Katarzyna Nierzwicka 1, Piotr Czauderna 1, Barbara Skoczylas-Stoba 2, Stefan Popadiuk 3, Jolanta Bonar 4, Krystyna Sawicz-Birkowska 5, Hanna Kaczmarek-Kanold 6, Przemysław Małkowski 7, Krzysztof Kikutski 8, Wojciech Madziara 9, Anna Siktiewicz 11, Anna Szołkiewicz 12, Sabina Szymik-Kantorowicz 13, Violetta Œwitkiewicz 14

Abstract

Objectives: Hepatoblastoma (HB) and hepatocellular carcinomas (HCC) are most common of all primary malignant liver tumors in children. This series of HB and HCC represents a preliminary report of treatment in 11 Polish pediatric oncology centers. Treatment protocols were based on two consecutive SIOPEL group studies: 2 and 3. Material and methods: From 1998 to 2002 there were 34 children with HB and HCC diagnosed (28 were HB and 6 were HCC). All HB and HCC patients received preoperative chemotherapy based on SIOPEL 3 protocols (CDDP alone, PLADO or SuperPLADO). Among HB 23 tumors were unifocal and 5 multifocal. Eight HB cases were qualified to the high risk group according to the SIOPEL protocol definition. Alpha-fetoprotein (AFP) was elevated in all cases. Lung metastases were present in one HB and in three HCC cases. In 5 cases diagnosis was made on the clinical ground, in as much as the patients initial biopsy was performed. Standard risk tumor were treated either Cisplatin monotherapy or PLADO regimen. High risk group was treated with SuperPLADO regimen. Twenty-one HB cases were operated in a delayed setting. Five tumor resections were incomplete: 3 macroscopically and 2 microscopically. In 4 cases information on surgery is missing. Results: Among 28 HB patient 19 are alive with no evidence of disease, 2 are alive with disease, 6 children died and one is lost to follow-up. Thus 3-year overall survival of HB patient was 75%, while 3-year event-free-survival was 64%. Median follow-up time is 40 months. Overall survival among standard risk group was 90% in comparison with 66% in the high risk group. All children with macroscopic residuum post resection died. Overall survival in HCC cases at 3 years is 33%, but of the two alive patients one currently experienced recurrence. Conclusions: Reported treatment for hepatoblastoma are similar to those achieved by other study groups and constitute an improvement in comparison with our retrospective series (OS=75% vs. 44%). High risk HB patients require modification of the current treatment strategy due to inferior survival than in standard risk HB. Treatment results of HCC (OS=33%) were clearly inferior to HB being in line with experience of other international study groups. Importance of the complete tumor resection, both in HB and HCC was confirmed.

Keywords: children, hepatoblastoma, hepatocellular carcinoma, surgery, treatment

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Introduction

In the last 15 years a steady increase in survival in pediatric liver tumors has been noted. First observations on improvement in survival with the use of adjuvant chemotherapy came from Children’s Cancer Study Group (CCSG) and Pediatric Oncology Group (POG) in the late 70’s. Later in unresectable cases neoadjuvant chemotherapy was applied [3, 9]. In 1986 Belgian and Canadian centers introduced in hepatoblastoma standard pre- and postoperative chemotherapy consisting of cisplatin and adriamycin. This chemotherapy protocol, called later PLADO, has become a golden standard of hepatoblastoma (HB) treatment in many centers, including Mother and Child Memorial Hospital in Warsaw. Efficacy of further modified protocols was tested by the multinational SIOPEL group in three subsequent trials: SIOPEL 1-3 [9-11]. It was shown that cisplatin alone is as effective as PLADO in standard risk HB [9, 10].

Despite chemotherapy progress complete tumor resection remains a goal of treatment and prerequisite for cure. If it cannot be achieved it is better to abandon any surgical attempt and resort to another treatment modality and/or consult the case with referral center. Our remarks on principles of the surgical treatment of liver tumors were presented during Surgical Workshop in Szklarska Poręba, Poland in 2002 [13]. It was reported that progress in surgical management of liver tumors had been associated with better knowledge of its anatomy, improved surgical technique and equipment (ultrasonic and water-jet dissectors, argon beam coagulation, thrombostatic materials), as well as new imaging techniques, new method of anesthesia and improved perioperative care.

Presented results emerged from multicenter cooperation of 13 Polish institutions within the study devoted to surgical treatment of malignant epithelial tumors of childhood including patients’ stratification according to risk groups in cooperation with the International Childhood Liver Tumors Strategy Group (SIOPEL). This study is coordinated in Poland by the Department of Pediatric Surgery of the Medical University of Gdansk.

Material and methods

In the period 1998-2002 all together 34 cases of primary epithelial liver tumors were treated in 11 Polish paediatric oncology centers. Twenty-eight were HB and 6 were HCC including one transitional (HB/HCC) liver tumor. Patients’ characteristics and stage are shown in Tab.1 and 2. Maximal tumor diameter ranged from 5 to 18 cm. Among HB 23 tumors (82%) were unifocal and 6 – multifocal. In HCC multifocal tumors had been associated with better knowledge of its anatomy, improved surgical technique and equipment (ultrasonic and water-jet dissectors, argon beam coagulation, thrombostatic materials), as well as new imaging techniques, new method of anesthesia and improved perioperative care.

Presented results emerged from multicenter cooperation of 13 Polish institutions within the study devoted to surgical treatment of malignant epithelial tumors of childhood including patients’ stratification according to risk groups in cooperation with the International Childhood Liver Tumors Strategy Group (SIOPEL). This study is coordinated in Poland by the Department of Pediatric Surgery of the Medical University of Gdansk.

Treatment protocols were based on consecutive SIOPEL studies: SIOPEL 2 and 3. Standard imaging methods included: abdominal ultrasonography (US), chest X-ray (AP and lateral), computed tomography (CT) of the abdomen (with and without i.v. contrast) and chest (to detect eventual pulmonary metastases) and/or magnetic resonance imaging (MRI). Also complete blood count (including platelet level) and serum AFP were obtained at diagnosis. AFP, if elevated, was used to monitor response to treatment. The protocol required preoperative assessment of tumor extent according to PRETEXT classification, which is described in details elsewhere [12]. Closed needle biopsy was performed in 2 cases, open biopsies were done in 21 cases (18 – wedge and 3 – open needle biopsies). In one case liver biopsy was done laparoscopically. In 5 cases diagnosis was made on the clinical ground. In 4 patients data on biopsy are missing. Patients with biopsy proven HB and HCC were qualified to two risk groups on the basis of PRETEXT grouping. High risk tumors were those PRETEXT 4 (involving the whole liver) or belonging to any PRETEXT category with the following features: significant intravascular involvement (V or P), extrhepatic extension and/or distant metastases (M). Hence high risk tumors were primarily unresectable. All others formed standard risk group. In tumors occurring between 6 months and 3 years of age with unequivocal imaging and increased AFP level diagnosis of hepatoblastoma on clinical ground was allowed. Before definite surgery Doppler US and helical CT were required. All patients with hepatoblastoma received preoperative chemotherapy which was dependent on the risk group assignment. Standard risk tumors were treated either cisplatin monotherapy or PLADO regimen (those registered in the SIOPEL 3 trial were randomized), however few patients were not randomized. High risk patients were treated with SuperPLADO regimen, which included 2-weekly cisplatin alternating with doxorubicin administered together with carboplatin. Four tri-weekly chemotherapy courses were given. Postoperatively two more courses of the same chemotherapy were administered. For details of the treatment protocol check elsewhere [9, 10, 11].

Cisplatin alone was used in 10 children, PLADO in 7 cases and SuperPLADO in 9 cases. In 2 cases details on preoperative chemotherapy are missing. Twenty-one HB cases were operated in a delayed setting. Type and completeness of performed surgery is shown in Table 3. Treatment of operable hepatocellular carcinoma (HCC) patients was started with tumor resection followed by 6 postoperative courses of SuperPLADO regimen. Two HCC cases were primarily operated including one orthotopic liver transplantation Treatment of unresectable and/or metastatic HCC was identical with high risk HB. One child with HCC was operated in a delayed setting after partial response to preoperative chemotherapy. In non-responders (2 cases) chemoembolization was used.
Results

Among 28 HB patients 19 are alive with no evidence of disease, 2 are alive with disease, 6 children died and one is lost to follow-up. Thus 3-year overall survival of hepatoblastoma patients was 75%, while 3-year event-free-survival was 64%. Three deaths resulted from disease progression and 3 from treatment complications: one was surgery-related (intestinal necrosis), one resulted from chemotherapy complications (severe neutropenia and typhlitis) and one was unexplained (possible anthracycline-related cardiomyopathy). Median follow-up time is 40 months (range from 24 to 72 months). Overall survival among standard risk patients was 90% (17 out of 19) in comparison with 66% (6 out of 9) in the high risk group.

Preliminary analysis showed that 75% of standard risk patients (12 out of 16; in 3 cases data are not known) responded to preoperative chemotherapy (CDDP or PLADO), while from the high risk group 55% (5 out of 9) responded to SuperPLADO protocol.

All together in HB cases 5 incomplete tumor resections were done: in 3 macroscopic residuum resulted and in two microscopic residuum was left. All patients with macroscopic residuum died, while one patients after microscopically incomplete resection is alive with no evidence of disease while another died due to surgical complications. In 4 cases information on surgery is missing.

Overall survival in HCC cases at 3 years is 33% but one of the two alive patients currently experienced recurrence. Among 6 patients with HCC in 2 cases primary tumor resection was done (including one orthotopic liver transplantation) was performed and 3 patients were treated with induction chemotherapy. In 2 non-responders chemombolization was used. Two patients were never made operable and in one data on surgery are missing.

Table 1

<table>
<thead>
<tr>
<th>Localisation</th>
<th>Hepatoblastoma</th>
<th>Hepatocellular carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both lobes</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Right lobe</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Left lobe</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>PRETEXT</th>
<th>Hepatoblastoma</th>
<th>Hepatocellular carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>III</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

Discussion

Reported treatment results for HB are similar to those achieved by other study groups (OS=75%). Introduction of efficient chemotherapy has contributed to a significant progress in the treatment of hepatoblastoma as nowadays overall survival reaches 70%. Before this era only 25% of HB patients survived and tumor resection was possible in not more than 50% of patients, while half of them died later due to metastases [3]. Similarly retrospective analysis of the Polish treatment results for the period 1985-1995 showed inferior outcome with only 44% long term survival in hepatoblastoma due to the scarcely used preoperative chemotherapy and few extended liver resections done [1, 6]. In that period as many as 30% of analyzed tumors were never made operable. Thus, currently achieved treatment results of hepatoblastoma in Poland constitute a significant improvement in comparison with our past series [1]. However despite overall improvement prognosis of the high risk patients remains uncertain – with 66% overall survival vs. 90% for the standard risk group. Similar results were achieved in the SIOPEN 2 study, in which 3-year event-free-survival for high risk patients was 47% vs. 89% for standard risk [9, 10]. Most failures resulted from tumor’s unresectability and insufficient or complete lack of response to preoperative chemotherapy. This confirms that high risk patients require some modifications in treatment strategy, which should be aimed at increase of resection rate. This includes more efficient induction chemotherapy, as well as more frequent use of liver transplantations in hepatoblastoma cases unresectable by conventional means [7, 8].

Treatment results of hepatocellular carcinoma (33% overall survival) were clearly inferior to hepatoblastoma is confirmed by most international study groups, in which survival remained in the range of 20-30% [2, 5]. Complete tumor resection should be an essential treatment step, both in HB and HCC. In the reported series all patients after macroscopically incomplete tumor resections died, while all HB cases, who underwent complete resection, had been alive and well. Due to the relatively short follow-up of some patients, incomplete data and limited number of cases presented analysis has a preliminary character only.

Conclusions

1. Overall survival achieved in hepatoblastoma – 75%, as well as EFS = 64% are similar to other international reports and constitute a significant improvement in comparison with the Polish past series (1985-1995): OS = 44%.
2. Complete tumor resection remains an essential step to achieve cure in primary epithelial liver tumors (HB and HCC). It is however difficult in high risk HB, as well as in HCC patients.
3. High risk HB patients require modification of the current treatment strategy due to inferior survival than in standard risk HB.
4. HCC are associated with poor prognosis and require completely different therapeutic strategy than HB.
Table 3
Characteristics of surgical treatment

<table>
<thead>
<tr>
<th>Type of surgery</th>
<th>Hepatoblastoma</th>
<th>Hepatocarcinoma</th>
<th>Radicality</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT Hemihepatectomy</td>
<td>9</td>
<td>1</td>
<td>7 rad. / 2 non-rad.</td>
</tr>
<tr>
<td>LT Hemihepatectomy</td>
<td>3</td>
<td>-</td>
<td>3 rad.</td>
</tr>
<tr>
<td>Extended Hemihepatectomy</td>
<td>7</td>
<td>-</td>
<td>5 rad. / 2 non-rad.</td>
</tr>
<tr>
<td>Atypical</td>
<td>3</td>
<td>-</td>
<td>2 rad. / 1 non-rad.</td>
</tr>
<tr>
<td>Central resection</td>
<td>1</td>
<td>-</td>
<td>1 rad.</td>
</tr>
<tr>
<td>Liver transplantation</td>
<td>-</td>
<td>1</td>
<td>1 rad.</td>
</tr>
<tr>
<td>Never operable</td>
<td>1</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Missing data</td>
<td>4</td>
<td>1</td>
<td>-</td>
</tr>
</tbody>
</table>

References
Liver transplantation for primary malignant liver tumors in children - single center experience

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Abstract

The aim of the study was to analyse results of treatment of primary liver tumors in children in a center performing the liver transplant program. Clinical material consisted of 42 children treated for hepatoblastoma or hepatocarcinoma since 1990, since liver transplant program was established. Retrospective comparison of results was performed according to type of the tumor and different treatment protocols. Survival of patients with hepatoblastoma is similar after primary conventional treatment (chemotherapy and resection) and primary transplantation after chemotherapy, however number of transplanted patients is too low to draw any conclusions. Patients with tumor recurrence after resection probably should not be qualified for transplantation as their prognosis is very poor. In hepatocarcinoma group overall results after chemotherapy and primary transplantation seem to be much better than after conventional therapy. This observation should be confirmed however with longer follow-up of these patients. In conclusion liver transplantation should be considered as an option early in the process of treatment of primary malignant tumors in children particularly with hepatocarcinoma, and transplantation as a primary surgical treatment in all patients with unresectable or marginally resectable tumors and without extrahepatic extension.

Key words: children, hepatoblastoma, hepatocellular carcinoma, liver transplantation, primary liver tumors

Introduction

Primary hepatic malignancies are main indication for liver transplantation in 2-4% pediatric recipients. This is well less than in adult population, where patients with hepatocellular carcinoma account for 11-12% of liver transplantations [3-5]. In contrast to adult group, there are no established strict criteria for qualification for transplantation in children with primary liver tumors, particularly that various types of hepatic tumors may develop in childhood (hepatoblastoma, hepatocarcinoma, undifferentiated sarcoma, angiosarcoma etc.) [1, 7, 12]. The aim of this study is to summarize our experience with this particular group of liver transplant recipients.

Material and methods

Liver transplant program was initiated in our institution in march 1990. Since then 222 liver transplants were performed till July 2004. We have analysed retrospectively data of 42 children treated for hepatoblastoma (HBL) or hepatocarcinoma (HCC) within the same period in our institution.

Patients with HBL

Between 1990 and 2004, 23 patients aged 5 months to 16 years, most with advanced tumors, were treated for HBL. Twenty children with HBL were treated initially with chemotherapy and then by conventional surgery – partial liver resection. In two of them recurrence was suspected despite of mult-
tiple postoperative chemotherapy courses, and were qualified for total hepatectomy of the remaining liver and orthotopic liver transplantation. Another 2 children were transplanted immediately after initial chemotherapy. In 1 patient only palliative treatment (chemotherapy) was administered.

Patients with HCC
There were 19 patients aged 3 to 18 years treated for HCC. In 9 children initial chemotherapy was administered, followed by partial liver resection, in 2 of them despite multiple resections tumor recurred and salvage transplantation was done in both of them. In 5 children liver transplantation was performed as primary surgical treatment. All of them exceeded Milano criteria for transplantation in patients with HCC. Initial chemotherapy was given in 4 of them. In 4 pts liver transplantation was performed due to suspected tumor development in cirrhotic liver (on the basis of CT or ultrasound findings or growing serum alphafetoprotein concentration). No pretransplant chemotherapy was administered to these patients. In 1 child only palliative chemotherapy was given.

Results

Hepatoblastoma
Twelve out of 23 (52.2%) children with HBL are alive and without active disease, while 11 patients died (47.8%). Best results were obtained with standard therapy consisting of chemotherapy, tumor resection and postoperative chemotherapy with long term survival of 11/20 pts (55.0%), including two patients who died after transplantation performed due to tumor recurrence. Among 2 patients who were transplanted primarily - 1 died 6 months after transplantation due to posttransplant lymphoproliferative disease. Another one is alive and well 25 months after Tx. Two additional children transplanted for tumor recurrence after previous resections died of metastatic disease despite of posttransplant chemotherapy. No graft involvement was observed in these patients (Fig. 1).

Hepatocellular carcinoma
Overall survival among 19 children with HCC is 12/19 (63.2%), 42.9% (3/7) children are alive and free of tumor after CHT and resection, while all other died of tumor recurrence. One patient is alive after extensive resection, however with poor prognosis due to extrahepatic invasion of the tumor at the time of surgery.

Among patients transplanted, 9/11 (81.8%) are alive, while 2 deaths were not related to tumor recurrence (early graft failure in 1 and chronic rejection in 1 (Fig. 2).

In all 4 patients with cirrhosis and suspected tumor small HCC was found in explanted liver. One of these patients died of graft primary non-function. One of two patients who underwent multiple resections before transplantation presented pulmonary metastases almost 2 years after transplantation and extensive metastasectomy was performed, however prognosis for this patient is rather poor. No recurrence or metastatic dissemination was noted until now among 5 patients who underwent primary liver transplantation after total hepatectomy.

Fig. 2 Hepatocarcinoma - actual results according to type of treatment (1990-2004)
Posttransplant observation is still relatively short in survivors (range: 5 - 40 months). AFP concentration remains within normal limits in all, however it is necessary to say that it was not elevated in 2 of 11 before transplantation (Fig. 3). Chemotherapy (1-3 courses) was given after transplantation to 5 patients. It had to be stopped in most of them after 1-2 courses due to complications. Liver graft function is good in all but one patient who developed severe cholangitis after chemotherapy, further complicated by intrahepatic bile duct strictures.

Fig. 3 Alphafetoprotein (AFP) in 9 patients with HCC after liver transplantation (follow up 5-40 months)
Discussion

Total hepatectomy with liver transplantation became an important therapeutic option for children with malignant primary hepatic tumors [1, 5, 9, 12]. Overall long term survival in pediatric liver transplant recipients is now reaching in leading centers 90-95% independently on indication for transplantation, while survival of children with hepatoblastoma and hepatocarcinoma after conventional treatment (chemotherapy + resection) is much lower, 60-80% and 30-40% respectively [2, 4, 5, 11, 12]. Various protocols and trials have been tried to improve results of conventional treatment, and much success was achieved independently by SIOP group, German group or Japanese group, mostly in HBL, with less optimistic results in patients with HCC [9, 10]. The place of total hepatectomy and liver transplantation have been not clearly defined in pediatric population, although many patients benefited from this type of treatment on the individual qualification basis [1, 9, 12].

Standard qualification criteria for transplantation in patients with primary hepatic tumors included in most centers: unresectable tumor, no extrahepatic tumor extension, no distant metastases (or in case of hepatoblastoma pulmonary metastases which cleared with chemotherapy), [5, 9]. It is obvious that conventional complex treatment is best for most patients with hepatoblastoma, however in our opinion transplantation should be considered as first surgery in these children in which tumor is unresectable, but also in cases of successful response of large, unresectable tumors to chemotherapy which allows downstaging of the tumor and complete extensive but marginal resection. Although not all agree that narrow margin free of HBL in resected liver specimen is important for long term survival, but one should keep in mind that recurrence of HBL in these patients indicates almost uniformly poor prognosis also after transplantation [2, 5, 9, 12].

Conventional treatment can not offer such good results in children with hepatocarcinoma despite intensive chemotherapy and improvements in resection techniques. In adult population large groups of patients with HCC were qualified to liver transplantation since 25 years, however no criteria for transplantation in these patients existed until study done by Mazzaferro et al in 1996 [7, 8]. They defined so called Milano criteria which have been accepted by most adult transplant centers. According to these criteria liver transplantation should be offered to patients with: one single tumor with diameter less than 5 cm, maximum number of 3 tumors with diameters less than 3 cm each no vascular invasion and no extrahepatic metastases. With these criteria 4 years survival after liver transplantation in adults achieve 75%, which is impossible to achieve by conventional treatment. One can not directly transfer Milano criteria to pediatric population, as tumor behaviour and response to chemotherapy in children are different in children and adults. There are no large enough groups to perform such analyses in pediatric patients however. Since all our patients with “non-accidentaloma” did not fit into Milano criteria one should expect very early tumor recurrence and dissemination in patients on immunosuppression. We did not observe such events in any patient till now with medium length follow-up. Multicenter analysis should be performed probably to try to define pediatric criteria for liver transplantation in patients with HCC, as single center can not collect number of patients necessary for such analysis.

In conclusion, liver transplantation should be considered as an option early in the process of treatment of primary malignant tumors in children, particularly with hepatocarcinoma, and transplantation should be considered as a primary surgical treatment in all patients with unresectable or marginally resectable tumors and without extrahepatic extension. Living related donor transplantation is one of important solution in best timing of transplantation within treatment protocol [6].

References

Hepatocyte ultrastructure in non-hemolytic hyperbilirubinemias

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Abstract

The non-hemolytic hyperbilirubinemias comprise a group of syndromes that are clinically and some in cases biochemically and genetically well characterized, and ultrastructural investigations of biopsy samples have still diagnostic applications. In this study ultrastructural analysis of biopsy tissues in order to identify pattern of features characteristic for different non-hemolytic hyperbilirubinemias among group of patients with clinical presentation of hyperbilirubinemia and normal histopathology were done. A group of 62 patients was selected on the basis of mild unconjugated hyperbilirubinemia and, in some cases, on the basis of a mild unconjugated or conjugated hyperbilirubinemia in the presence of repeatedly normal liver function tests and absence of hyperhemolysis. Electron microscopic investigation distinguished between patients in three groups of primary hyperbilirubinemia: Gilbert syndrome (21 pts), Dubin-Johnson syndrome (5 pts), Rotor syndrome (3 pts) and a group with non specific changes (33 pts). The ultrastructural features of hepatocytes that univocally distinguish primary hyperbilirubinemias from secondary diseases were presented. Electron microscopic methods permit on recognition of non-specific changes in the ultrastructure of liver tissue among samples obtained from patients with non-hemolytic hyperbilirubinemias and can be still usefull in diagnosis of jaundice.

Key words: congenital hyperbilirubinemia, Dubin-Johnson disease, Gilbert disease, Rotor disease, ultrastructure

Introduction

Idiopathic hyperbilirubinemias may encompass several primary and secondary diseases of bilirubin metabolism [2]. Bilirubin, an oxidative product of the heme group of hemoglobin, myoglobin and cytochrome P-450, in the liver is conjugated with glucuronic acid and than excreted in bile via ATP-dependent transporter [5]. Currently, injuries of molecular bases of this metabolic pathway are known [5]. Although, this knowledge allow for precision diagnosis of non-hemolytic hyperbilirubinemias in most cases, but still ultrastructural investigation of biopsy samples have diagnostic application. Clinically, conjugated and unconjugated hyperbilirubinemia can be distinguished on the basis of blood bilirubin level, normal liver tests and exclusion of hemolytic disease. In conjugated hyperbilirubinemias i.e Gilbert, Crigler-Najjar syndrome, transport of bilirubin from plasma into liver cells is impaired, while in conjugated hyperbilirubinemias, i.e Dubin-Johnson, Rotor syndrome, microsomal enzymes transferase, bilirubin transport from hepatic cells into bile canaliculi system are disturbed [2, 5]. In non-hemolytic hyperbilirubinemias, liver histopathology may present normal feature or cholestasis.

These investigations were aimed to identify ultrastructural pattern of characteristic features for different non-hemolytic hyperbilirubinemias among group of patients with clinical presentation of hyperbilirubinemia and normal histopathology.

Material and methods

Material

62 biopsy samples from patients (age 6-17 years, mean 10.2 ± 4.6) with marked liver disfunction and clinical symptoms of non-hemolytic hyperbilirubinemia were selected for electron microscopic studies among samples collected in the registry of Pathology Department of CMHI. Tissue samples were routinely preserved for histological, histochemical and ultrastructural examination. All of the examined patients had total bilirubin in the range of 1.3-5.1 mg% (Table 1).
Table 1
Total and direct bilirubin level in serum in distinguished groups of primary hyperbilirubinemia and with non-specific changes

<table>
<thead>
<tr>
<th>Disease</th>
<th>Number of patients</th>
<th>Age (years)</th>
<th>Total Bilirubin (mg%)</th>
<th>Average</th>
<th>Direct</th>
<th>Avs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gilbert Compl.</td>
<td>10</td>
<td>10-17</td>
<td>1,5-4,6</td>
<td>2,56</td>
<td>0,5-0,9</td>
<td>0,62</td>
</tr>
<tr>
<td>Incompl.</td>
<td>11</td>
<td>6-7</td>
<td>1,2-5,1</td>
<td>2,48</td>
<td>0,3-1,1</td>
<td>0,95</td>
</tr>
<tr>
<td>Dubin-Johnson</td>
<td>5</td>
<td>9-17</td>
<td>1,3-4,7</td>
<td>2,54</td>
<td>0,6-2,0</td>
<td>1,05</td>
</tr>
<tr>
<td>Rotor</td>
<td>3</td>
<td>16-17</td>
<td>1,6-4,5</td>
<td>2,10</td>
<td>0,4-0,8</td>
<td>0,55</td>
</tr>
<tr>
<td>Non specific</td>
<td>33</td>
<td>7-17</td>
<td>1,2-5,7</td>
<td>2,62</td>
<td>0,2-2,8</td>
<td>1,45</td>
</tr>
</tbody>
</table>

**Histology**

Five mm thick paraffin sections were stained in a routine manner with hematoxylin-eosin, azan, silver, PAS, PAS with diastase digestion and than reviewed to exclude other disease than hyperbilirubinemia.

**Electron microscopy**

Samples were fixed in 2.5% glutaraldehyde in cacodylate buffer at pH 7.3, postfixed in 2% OsO4, dehydrated in alcohols and embedded in Epon 812. Thick sections stained with toluidine blue were analysed under light microscope. Ultrathin sections stained with uranyl acetate and lead citrate were examined by electron microscopy.

**Results**

Examinations of paraffin and semi-thin sections showed in all cases an apparently normal liver, except for Dubin-Johnson that shown the existence of dark pigment predominantly in centrilobular areas.

On the basis of EM analysis morphological equivalents of primary congenital hyperbilirubinemias were confirmed in 29 cases and secondary reactive symptomatic hyperbilirubinemias were recognized in 33 cases.

**Gilbert syndrome**

Gilbert syndrome was diagnosed in 21 cases. There were observed shortening and mal-developed vascular pole microvilli or loss of microvilli, and increase of collagen in the Disse space in various intensity. In all patients an increase of the endoplasmic reticulum profiles with coexisting dilatation of rough endoplasmic reticulum with degranulation and pigment granules variable in size containing both electron dense material which alternate with less dense material and lipid droplets was observed (Fig. 1A). In 10 biopsy samples enlarged or giant, oval or polymorphic mitochondria with paracristalline inclusions (Fig. 1B) were present. In all cases the biliary pole of hepatocytes shown normal caniculi and microvilli, but with numerous pigment granules around. Lipofuscin granules were common finding. The remaining group of 11 patients showed uncompleted ultrastructural features of Gilbert disease, i.e. lack of giant mitochondria and no paracristalline in mitochondrial matrix.

**Dubin-Johnson syndrome**

Dubin-Johnson syndrome was defined in 5 cases. There were not seen alterations in the vascular pole of hepatocytes, which exhibit normal microvilli. Slightly increased number of smooth endoplasmic reticulum profiles that formed small vesicles, normal mitochondria with some variation in size, well developed Golgi profiles located around bile canicules were common features. Dilation of bile caniculi with loss of microvilli and characteristic pigment granules around were seen (Fig. 2). Pigment granules were composed mainly of granular material, however vesicle-like or lipid components were also seen. They were polygonal in shape and delineated by a single membrane. The lack of lipofuscin in hepatocytes was common feature.

Fig. 1 Gilbert syndrome: (A) vascular pole of hepatocyte with single microvilli. Mag. x 40 000, (B) mitochondrium containing paracristallines, mag. x 22 000
Rotor Syndrome

Rotor syndrome was recognised in 3 cases. Only slight changes in hepatocytes were observed: some of the bile canicules were dilated and exhibited reduced number of microvilli, the interhepatocytic junctional interspaces were dilatated and cell membranes along the lateral cell surface formed microvilli (Fig. 3a), cell membranes facing the Disse space showed reduced number of microvilli. In side of hepatocytes a few pleomorphic mitochondria or megamitochondria with paracrystalline were seen. The most characteristic feature was lack of lipid component in pigment granule that composed of electron dense and less dense granular material. They were distributed mainly around bile canicules (Fig. 3b).

Non characteristic lesions

Non characteristic ultrastructural fine lesions accompanying secondary hyperbilirubinemia were found in 33 cases. Reactive changes were recognized in 9 cases. There were increased number and elongation of microvilli on the vascular pool (Fig. 4), polymorphic mitochondria (sometimes with paracrystalline inclusions), increased number of smooth endoplasmic reticulum profiles, the presence of single lipid droplets and pigment or lipofuscin granules in cytoplasm, and collagen fibrils in Disse spaces.

Discussion

In about 50% of the patients with clinical symptoms of hyperbilirubinemia electron microscopy confirmed primary congenital hyperbilirubinemia. Among of them, complete ultrastructural features of Gilbert syndrome were presented in 10 cases, uncomplete Gilbert in 11 cases, Dubin-Johnson in 5 cases and Rotor in 3 cases.

In seventies the ultrastructural observations were helpful to establish the morphological equivalent of primary hyperbilirubinemia and distinguish combination of defects impairing primary hyperbilirubinemas, particularly in Gilbert syndrome when complete and incomplete features coexisted [1, 4, 6]. Our patients with Gilbert disease clinically were characterized by relatively low-grade direct bilirubin and they reached a higher proportion of unconjugated bilirubin. These abnormalities were correlated with decrease or the lack of microvilli at sinusoidal pole.

It is known that the liver in Dubin-Johnson syndrome might be characterized by slight and inconsistant changes. In our patients, the canicular pole exhibited normal microvilli or disappearing microvilli coexisting with a slightly dilated lumen. These results suggest that disturbances of bile flow were caused by different disfunctions of the canicular area. These are in agreement of with distinct mechanism of bilirubin glucuronide transport involving ATP-dependent and ATP-independent system that have been proposed [7].
Patients with Rotor syndrome ultrastructurally presented fairly similar features to Gilbert and to Dubin-Johnson simultaneously: some injury of vascular and canicular pole concomitant with an increase of smooth endoplasmic reticulum and pleomorphic mitochondria in hepatocytes. These similarities between some ultrastructural features of Gilbert and Dubin-Johnson syndrome were signaled in literature before [8]. The presence of microvilli along the lateral surface of hepatocytes was a particular feature, which distinguished patients with Rotor syndrome from other patients. Patients with Rotor syndrome exhibited lower level of direct bilirubin than unconjugated, and this observation does not agree with main idea of liver defect [5]. However, similar data on a direct bilirubin in serum have been mentioned in literature [3].

Electron microscopic methods permit on recognition of non-specific changes in the ultrastructure of the liver tissue among samples obtained from patients with non-hemolytic hyperbilirubinemias and can be still usefull in diagnosis of jaundince.

References

Congenital Heart Diseases Database: analysis of occurrence, diagnostics and coexistence with anomalies of other systems. Single institution experience

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Abstract

The Congenital Heart Diseases Database was created in the Children’s Memorial Health Institute during the period of 2001 – 2003. It contains over 1000 preparations of congenital heart defects of children aged from 0 till 14 years. According to our data, the most common defect was ventricular septal defect. The compatibility of the clinic and pathological diagnoses was high (about 80% of the diagnoses were confirmed), however significantly higher after 1990. Although in our Database plenty of coexisting anomalies and diseases of the other systems or organs occurred, we found no significant relations between them and congenital heart defects.

Key words: congenital heart disease, database, heart defects

Introduction

Since 1980 until now, the Children’s Memorial Health Institute of Warsaw has assembled over 1200 congenital heart defects preparations from patients aged 0-14 years. Between September 2001 and March 2003 we have made the repeated sections of those preparations and created “The Congenital Heart Diseases Database” (CHDDb) that includes 1037 records with full descriptions of the hearts’ and defects’ anatomy, clinical and sectional diagnoses, anomalies of other systems and performed operations. This is to present the direct conclusions that have arisen from this Database, including the frequency of occurrence of the particular heart defects, compatibility of the clinical and autopsy diagnoses and finally, regularity in coexistence of the congenital heart defects with the anomalies of the other systems.

Material and methods

The Congenital Heart Diseases Database is composed of 1037 preparations of the hearts with the congenital defects. They are fully described in four files which contain personal patients’ data, clinical diagnoses with the descriptions of defects of the other systems and types of performed operations, pathologic diagnoses of the heart defect and coexisting diseases. The last file shows complete measurement of all the hearts including external dimensions as well as internal measurements of the valves, arteries, veins or myocardium.

The patients’ age was between 1 day and 14 years. There were 437 female and 600 male patients described and they were all admitted to Children’s Memorial Health Institute during the period 1980 – 2003 and almost half of them were operated because of the heart defect.

A nomenclature for the congenital heart disease was based on the International Nomenclature for Congenital Heart Surgery that was officially adopted at the Annual Meeting of the EACTS in Glasgow on September 6, 1999 [3].

Results and discussion

Occurrence of the congenital heart defects

The most common anomaly that appeared in our material was ventricular septal defect (VSD) with the frequency just under 30% (Fig.1). In details, there were 31 cases of VSD in muscular part of the septum (VSD single–29; VSD multiple-2 cases) and 259 cases of this defect in other parts of the interventricular septum (VSD; NOS).
The frequency of patent arterial duct (PDA) was about 19%. Similarly, other congenital heart defects like transposition of the great arteries (TGA) and atrial septal defect (ASD) are about 18%. Although there were over 160 ASD diagnosed, the largest group was due to persisted foramen ovale (PFO) type (50%). ASD secundum type appeared in 69 cases that was just over 40% of all atrial defects. Other atrial defects, like ASD type sinus venosus, coronary sinus type or single atrium, appeared rarely (14 cases).

Tetralogy of Fallot (TOF) was diagnosed in about 12% of all cases. Atrioventricular canal (AV canal) as well as coarctation of aorta (CoA) concerned 9% of all heart defects. Hypoplastic left heart syndrome (HLHS) likewise pulmonary atresia (PA) were visible in 7% of the examined preparations. Similarly hypoplastic right ventricle (HRV) or pulmonary stenosis (6,5%). Double outlet from the right ventricle (DORV) and total anomalous pulmonary venous connection (TAPVC) appeared in less than 5% of all cases. The rarest were aortic atresia (3 patients), aortic valve hypoplasia (1 patient), pseudotruncus (3 patients) and inversion of the ventricles (2 patients).

There is also some regularity with reference to the gender of the patients. The difference is most significant in the group of diseases like partial anomalous pulmonary venous connection or right ventricle outlet obstruction, which occurred two to five times more often in the female group than in the male group. However aortic atresia, Bland-White-Garland syndrome and cor triatriatum occurred in our material only in reference to the male group. Such defects like truncus arteriosus, aortic stenosis, HLHS, TGA or mitral atresia occurred over two times more often in the group of boys than in the group of girls.

Clinical and autopsy diagnosis

There are 1037 congenital heart diseases in the Database, however clinical diagnostics and diagnoses were established due to 867 patients. In this group, autopsy diagnoses differed from clinical diagnoses in about 19% (166 cases).

Clinical diagnostics was based on physical examination, ECG, blood pressure measurement, echocardiography and invasive cardiology, which was used from the beginning 1990. Because of that fact, we decided to divide our data into two groups: in the first group there were patients that had been admitted to the Children’s Memorial Health Institute till the end of 1989 (533 patients); the second group included rest of the patients (334 patients). The results of a comparison between clinical and pathologic diagnoses were as follows: 166 children (22%) from the first group were misdiagnosed during their hospitalization. In the second group, where the patients were additionally diagnosed by means of the invasive cardiology, the number of misdiagnoses has fallen to 15% (Table 1). Among 166 incorrectly diagnosed congenital heart defects, the most often was HLHS (about 13%), than TGA and AV canal (just under 9%). In contrast, the greatest conformity between clinical and autopsy was due to interrupted aortic arch (over 90%) and TAPVC or PDA (about 80%).

Table 1

<table>
<thead>
<tr>
<th>Period</th>
<th>Compatibility</th>
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<tbody>
<tr>
<td>1980-1989</td>
<td>78%</td>
</tr>
<tr>
<td>1990-2003</td>
<td>85%</td>
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Coexistence of the congenital heart defects with the anomalies of other systems

In reference to the CHDDb there were no significant relations between heart defects and other anomalies. The most common were malformations of the alimentary tract, polysplenia, asplenia, urinary tract defects and additional superior caval vein which occurred mainly in reference to heart defects like AV canal, coarctation of aorta, TAPVC and TOF. There were eight patients with situs inversus, although with different heart defects.

In the group of patients with chromosome aberrations there were 24 patients with Down syndrome. Over 62% of these patients suffered from atroioventricular canal and about 34% from VSD. There was one patient with Patau syndrome that had coarctation of aorta and one patient with Edward’s syndrome who suffered from VSD.

In our Database occurred six patients with developmental multiply malformations. Three patients with Pierre-Robin sequence suffered from ASD, VSD and TOF. Two children with DiGeorge syndrome had TOF and tricuspid valve atresia, while in literature, the most common are the anomalies of the aortic arch [1, 2]. The congenital heart disease of one patient with Noonan syndrome was AV canal (in literature, over 50% patients with Noonan syndrome suffer from pulmonary stenosis) [2].

References

Solid pseudopapillary tumor of pancreas.
Case report and the review of literature

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Abstract
Authors describe the case of 14 year-old girl operated on because of the neoplasm of tail of pancreas proved to be solid pseudopapillary pancreatic tumor. Radical resection was curative. After surgery she made uneventful recovery being symptoms’ free for 8 months. The review of literature on this rare, relatively low, malignant tumor is presented.

Key words: pancreas, pseudopapillary tumor, solid tumors

Introduction
Solid pseudopapillary tumors of pancreas (SPTP) first described by Frantz in 1959 are rare findings among other pancreatic tumors [5, 6, 9, 10, 11, 13]. To date a bit more then 500 cases have been described in the literature [16]. SPTP occurs predominantly in teenage girls and young women although all age groups may be affected and also males are not disease–free [12, 15, 17, 18]. The tumors are reckoned as a low-grade malignant papillary–cystic neoplasms of the pancreas of unclear histogenetic origin, but with highly characteristic, distinct histology and usually quite benign biologic behaviour [3, 9, 10, 13, 15, 18]. Their clinical presentation is vague and non characteristic. The most common symptoms are abdominal pains, nausea, emesis, sometimes palpable abdominal mass and jaundice – if the head of pancreas is involved [1]. Asymptomatome course is not uncommon and diagnosis can be established incidentally following blunt abdominal trauma [3, 9, 19]. Complete resection is usually curative and so the radical surgery is the treatment of choice. No adjuvant therapy is recommended.

Case report
Patient
Girl 14-year-old girl (J. P.; files 054774B) was transferred to Department of Pediatric Surgery, Polish Mother’s Health Institute from local hospital because of suspected pancreatic tumor. She gave the history of year-long, moderate, occasional abdominal pain and nausea and single incident of syncope just before admission to the hospital. Her general condition was quite good. Physical examination revealed palpable mass in upper abdomen on left side. No other abnormalities have been detected. Laboratory investigations were all within normal range. In abdominal ultrasonography a solid tumor in left upper quadrant, originating probably from pancreas tail and reaching spleen, was found. CT scan confirmed this finding (Fig. 1) showing the tumor of pancreas tail about 6 cm in diameter in the vicinity of spleen and its vessels. The indications for surgical treatment were established. Exposure of the pancreas revealed the presence of the well vascularized mass arising from the tail of pancreas and reaching to the hilum of the spleen. Main splenic vessels were surrounded by the tumor and could not be liberated. The resection of the tumor

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was performed “en bloc” with the spleen and distal part of the pancreas 1 cm from the borderline – within healthy tissue. The hemostasis was achieved and the line of resection of pancreatic tissue protected with continuous 4.0 nonabsorbable suture. No other abnormalities were detected in abdominal cavity and parietes were closed in layers.

Because of splenectomy that has been performed together with the tumor’s resection - vaccinations against Streptococcus pneumoniae, Haemophilus influenzae and Neisseria meningitidis were carried out. Now, eight months postoperatively girl is well, free of symptoms. The patient has made an uneventful recovery and was discharged home on 12th postoperative day.

**Histopathology**

The specimen consisted of tumor – 6 cm in diameter with margin of normal pancreatic tissue attached to the hilum of the spleen, without its infiltration, encompassing splenic vessels. The tumor was well demarcated, its architecture was partly solid and partly cystic. The solid part was built of uniform, medium-size cells with faintly eosinophilic cytoplasm and round nuclei. Mitoses were not numerous, but foci of increased mitotic activity were present as well. The tumor’s cells formed solid structures, cords and trabeculae (Fig. 2).

In the cystic part of the tumor there were foci of degeneration and haemorrhage resulting in creation of pseudocystic and pseudopapillary pattern, particularly around small vessels (Fig. 3). No vascular invasion was found. On the borderline of the tumor and pancreatic parenchyma a few otherwise normal glands were found with the lining partly consisting of tumor cells (Fig. 4). The tumor cells showed: positive immunostaining for α₁ antitrypsin (Fig. 5), chromogranin, vimentin, neuron – specific enolase (NSE) and synaptophysin. The final histopathologic diagnosis was - solid pseudopapillary tumor of pancreas (SPTP).

![Fig. 1 CT scan: tumor of the tail of pancreas](image1)

![Fig. 2 General architecture of the tumor - the solid part. Two mitoses are present. (HE 100x)](image2)

![Fig. 3 Pseudopapillary pattern, particularly around small vessels. (HE 100x)](image3)

![Fig. 4 Tumor’s cells within the lining of some glands. (HE 200x)](image4)

![Fig. 5 Positive immunostaining for alfa1 antitrypsin. (HE 100x)](image5)
Discussion

Preoperative diagnosis of pancreatic tumor, putting aside uncharacteristic clinical symptoms as far as its size, localization, detailed anatomy and topography is concerned, is based upon ultrasonography, computed tomography, magnetic resonance image [4, 7, 15, 17]. Still diagnostic failures, such as pseudocyst or ruptured hepatic mass are described in literature [17, 19]. Some authors advocate preoperative fine needle biopsy to establish exact histopathology of neoplasm [3, 17]. However such a policy is controversial as needle biopsy may not allow an undisputed diagnosis and the indications for operation exist, this way or another. This was a case in our patient, too. Aggressive surgery and complete resection produces the specimen to be meticulously examined [1, 5, 13, 15, 16]. In 1996 solid pseudopapillary tumor was introduced in WHO classification of tumors of the exocrine pancreas [8, 11]. It is usually included into cystic group of exocrine neoplasm of pancreas that encompasses [8, 20]:

- serous cystic neoplasms,
- mucinous cystic neoplasms,
- solid papillary neoplasms,
- intraductal pancreatic mucinous neoplasm.

Prognosis for the patients with these tumors is much better than that of patients with adenocarcinoma, although in about 5% of patients with SPTP malignant course with metastases and peritoneal spread can develop [2, 6]. It is estimated that after surgical resection the outcome is excellent resulting in long – term survival in about 90% of patients [5, 9, 15, 16, 18]. Complete radical resection may need extension to neighbouring organs e. g. transverse colon or spleen – as was a case in our patient. Recurrence has been described in approximately 10% of cases [2, 5, 10, 15]. Solid pseudopapillary tumor of pancreas should be always taken into consideration in the differential diagnosis of pancreatic neoplasm.

Acknowledgements

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References

Liver tumors of childhood - pathology. Report of the Kiel Pediatric Tumor Registry*

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Dear Colleagues,

Over the years I have collected a series of 522 primary liver tumors. Hepatoblastomas, accounting for 51.3% of the cases, are by far the most frequent liver tumors in the files of the Registry, followed by infantile hemangioendothelioma and hepatocellular carcinoma (15.5%, each). All other liver tumors, including mesenchymal hamartoma, focal nodular hyperplasia, embryonal rhabdomyosarcoma and undifferentiated sarcoma, are rare.

Hepatoblastoma has the peak incidence in the first two years of life, and up to 90% of the cases occur before the age of five years. As it is well known, the wide majority of hepatoblastomas present as a single mass involving in decreasing frequency the right lobe, both lobes and the left lobe. Approximately 20% of the cases are multifocal. Microscopically, the more frequent epithelial hepatoblastomas (56%) and the less frequent (44%) mixed epithelial and mesenchymal hepatoblastomas have to be distinguished. With regard to the subtypes mixed epithelial/mesenchymal tumors without teratoid features are most frequent (34%), followed by pure fetal (31%), fetal/embryonal (19%) and mixed teratoid hepatoblastomas (10%). The macrotrabecular and the small cell/anaplastic hepatoblastoma variants are very rare (3%, each). Notably, a pure embryonal variant of epithelial hepatoblastoma does virtually not exist.

Nevertheless, data from the Liver Tumor Study HB 94 show that the histologic subtype does not influence the prognosis significantly. By contrast, significant prognostic factors are the tumor growth pattern in the liver, vascular invasion, distant metastases, surgical radicality, initial levels of AFP, and the response to chemotherapy. The overall survival rate of patients with hepatoblastoma in different multicenter studies is approximately 75%. The prognostically much more unfavorable hepatocellular carcinoma (HCC) is significantly less frequent than hepatoblastoma except in countries with high hepatitis B virus infection rates. In the files of the Kiel Pediatric Tumor Registry 81 cases of HCC including 14 cases of the fibrolamellar variant are collected. The proportion of HCC to hepatoblastoma is 0.3:1. Most cases of HCC did occur clinically in the second decade of life, this in contrast to the wide majority of hepatoblastomas. Microscopically, pediatric HCC are similar to HCC of the adults. Usually the tumor cells are larger than the surrounding normal hepatocytes. The tumor cells can be arranged in a pseudoglandular, trabecular, or pseudoacinus pattern. In most cases the cytoplasm shows an intensive eosinophilia. Rarely HCC displays tumor cells with clear cytoplasm similar to clear-cell renal carcinoma and to some fetal-epithelial hepatoblastomas. Mitotic activity and nuclear atypia are variable from case to case and may be variable even in the same tumor. In this case at least moderate atypia and some mitotic figures can be seen. Immunohistochemically, tumor cells express AFP, and vessel invasions are very common. Since many HCC are hepatitis B virus (HBV)-associated, it is important to look for features of preceding HBV infections. The fibrolamellar variant of HCC is not associated with virus infections and, moreover, is not associated with other conditions like metabolic diseases or biliary atresia, which are well-known risk factors for the development of classic liver cell carcinoma. In our files 17% of the liver cell carcinomas belong to the fibrolamellar variant. Fibrolamellar carcinoma occurs predominantly in older children, adolescents and young adults and usually presents as a solitary, well-circumscribed tumor mass with a predilection to the left lobe. Microscopically, cords of relatively large tumor cells are separated by paucicellular collagen bands. The cytoplasm is intensively eosinophilic, and frequently contains lumina. The proliferation activity is comparably low indicating slow tumor growth. Late tumor relapses can occur, and in so far the final prognosis of fibrolamellar carcinoma is not so favorable as was supposed initially.

The most important mesenchymal tumors of the liver are infantile hemangioendothelioma, mesenchymal hamartoma, embryonal rhabdomyosarcoma, and undifferentiated (embryonal) sarcoma with 15.5%, 4.8%, 5.6%, and 2.9%, respectively, of the cases collected in our Registry. With regard to the age at clinical manifestation, significant differences between these tumors become overt. Thus, most cases of infantile hemangioendothelioma are diagnosed in very young children. Referring to our publication on tumors of the newborn and the very young infant [3], 86% of the

* The lecture was presented during the Meeting of the Polish Pediatric Group for the Solid Tumors’ Treatment (Liver Tumors), 22-24.04.2004, Gdansk, Poland
Favorable. Tumor relapses are rare. It may be impossible to exclude undifferentiated sarcoma of chylomic hamartoma is easy, except in small biopsies, in which to the very young age of the patients the diagnosis of mesenchymal hamartoma can be present at birth, like hemangiendothelioma. The wide majority of mesenchymal hamartomas occurs in patients under the age of 2 years. Embryonal rhabdomyosarcoma of the liver has a broad age distribution, but the majority of rhabdomyosarcomas develop within the first 5 years of life, this by contrast to the probably rhabdomyosarcoma-related undifferentiated (embryonal) sarcoma (malignant mesenchymoma of the liver in the old nomenclature), for which the peak incidence is between five and ten years of age. Infantile hemangiendothelioma occurs as a solitary mass or consists of multiple tumor nodes involving large parts of the liver. Under the microscope infantile hemangiendotheliomas are composed of multiple, sometimes dilated vascular channels lined by plump endothelial cells. Large or multicentric lesions can be complicated clinically by consumption coagulopathy or disseminated intravascular coagulation (Kasabach-Merritt-syndrome) or, due to intralobepal vascular anastomoses, by severe congestive heart failure. Most infantile hemangiendotheliomas have to be classified according to DEHNER’s proposal [2] as type I-hemangiendotheliomas. The rare type II-hemangiendothelioma shows a more distinct papillary pattern and endothelial cells with more atypical nuclei than in type I -tumors. Type II-infantile hemangiendotheliomas are now considered to be pediatric angiosarcomas, but malignant behavior of infantile hemangiendotheliomas is generally rare. The majority of the tumors are principally benign lesions. The prognosis depends on size and distribution of the tumor throughout the liver, on the severity of complications, and the resectability, which may be impossible in multifocal disease. Mesenchymal hamartomas are generally solitary, no encapsulated and can be very large. Up to 30% of the tumors are pedunculated. Typically, mesenchymal hamartomas show cysts containing a clear fluid. Microscopically, in typical cases a so-called ductal-plate pattern with tortuous bile ducts, surrounded by loose connective tissue, and at the periphery by a more dense fibrous tissue, can be seen, so that ductal-plates are reminiscent on fibrocystic disease of the female breast. Some bile ducts may show slight nuclear atypia. Fluid accumulations in the tumor tissue can appear with moderately dilated lumina. Immunohistochemically, the tumor cells express vimentin intermediate filaments. In addition, some tumor cells are positive for desmin too, but true rhabdomyoblasts, particularly more differentiated rhabdomyoblasts with cross striations do not occur, this in contrast to embryonal rhabdomyosarcoma. Nevertheless, the histologic features of undifferentiated sarcoma and embryonal rhabdomyosarcoma may overlap. However, basically, the undifferentiated sarcoma is a more primitive sarcoma than embryonal rhabdomyosarcoma. The prognosis of undifferentiated sarcoma of the liver was very poor in the past (mortality about 80% - STOCKER and ISHAK 1978). Introduction of multimodal soft-tissue sarcoma therapy strategies has improved the prognosis dramatically. According to data of the aforementioned Italian/German study [1] 12 from 17 patients (approximately 70 % of the cases) have survived with follow-up ranging from 2.4 to 20 years [1]. Liver tumor pathology covers a wide variety of malignant and benign, epithelial and mesenchymal tumor entities. The wide majority of principally epithelial tumors (hepatoblastomas and liver cell carcinomas) are malignant, whereas the majority of mesenchymal tumors (except undifferentiated sarcoma and embryonal rhabdomyosarcoma) are morphologically benign. The prognosis of malignant liver tumors has improved dramatically during the recent few decades. Improvement of prognosis is due to interdisciplinary cooperation in multimodal therapy studies. Another milestone was the development of new pediatric surgery strategies and techniques, which have contributed significantly to the prognostical success in malignant and in surgically problematic benign tumors of the liver too.

Embryonal rhabdomyosarcoma of the liver develops either in the extrahepatic bile ducts or intrahepatic. Occlusion of larger bile ducts induces obstruction jaundice. Microscopically, rhabdomyosarcomas of the liver are in most cases embryonal rhabdomyosarcomas. Particularly, they present as the botryoid variant of embryonal RMS exhibiting the characteristic subepithelial cambium layer. This layer consists of parallel to the surface arranged, at least moderately differentiated, intensively desmin-positive rhabdomyoblasts. This diagnostic cambium layer the botryoid RMS usually shows small stellate-like cells arranged in a loose pattern. By contrast to botryoid rhabdomyosarcomas of other locations, the surface of the tumor proliferations can be covered by cylindrical or cuboidal epithelial cells and not by metaplastic squamous epithelial cells. Treatment should be done according to the well-established soft-tissue sarcoma therapy protocols. The same is true for undifferentiated sarcoma of the liver. Undifferentiated (embryonal) sarcoma of the liver usually develops in children between five to ten years of life. The median age of 17 patients with undifferentiated sarcoma treated in a cooperative Italian/German study [1] was seven years. Macroscopically, this undifferentiated sarcoma (this specimen stems exceptionally from an adult patient) shows a grayish-white to red and brown cut surface with intensive necrosis. Under the microscope the tumor consists of spindle and seellate cells arranged within a myxoid stroma. In addition to these comparably small cells some larger and pleomorphic tumor cells can be seen. Typically, the periphery of the tumor shows entrapped bile ducts with slight nuclear atypia. These bile ducts can appear with moderately dilated lumina.