Histological classifications are essential for prognosis in children malignancies. Currently, the histological type of tumor is one of the main prognostic factors in this group. We investigated histoclinical features of nephroblastoma in relation to SIOP 93-01 and SIOP 2001 Classifications of Renal Tumors of Childhood. We examined all the routinely available histological features and histological nephroblastoma types and investigated their influence on patients’ survival with the use of log-rank test and Kaplan-Meier method. The results of statistical analysis indicated that SIOP 93-01 more precisely separated nephroblastoma types according to their biology and malignant potential. We also observed that epithelial type of nephroblastoma showed a mixture of results typical for both intermediate and high risk tumors. What is more, we noticed statistically important correlations between developmental defects found in patients with nephroblastoma and tumor volume and the course of disease.

Key words: Wilms’ tumor, histoclinical features, SIOP Protocols.

Introduction

Renal neoplasms are a prominent group of children malignancies. The most frequent kidney tumor in children is nephroblastoma (Wilms’ tumor) with an incidence of 1:10,000 of births. It constitutes 88% of tumors of kidney, 80% of neoplasms of the genitourinary tract, 8% of solid tumors and 3% of all children malignancies [1-3].

Sporadic (99%) and unilateral (93-94%) form of nephroblastoma are prevalent. 5% of cases of unilateral nephroblastoma are multifocal. In bilateral (6%) Wilms’ tumor group, 5% is synchronous, while 1% is metasynchronous. Predominance of nephroblastoma in females is observed (F: M 1:0.92 in unilateral and 1:0.6 in bilateral tumors) [4-6]. The first symptom of nephroblastoma in most cases is the presence of palpable or even visible abdominal mass. The condition of patients with Wilms’ tumor is usually good [7-11]. Anemia is the prevalent clinical symptom found in laboratory tests (50% of cases). Other symptoms observed in patients with nephroblastoma include: hypertension, pain, haematuria, constipations, elevated body temperature, lack of appetite and vomiting (seldom). The following symptoms sporadically accompany Wilms’ tumor: hepatocellular carcinoma [12], autoimmune neutropenia [13], erythrocytosis [14], urticaria [15] and the following syndromes: acquired von Willebrand’s syndrome [16], Pierre-Robin syndrome [17] and HPT-JT (hyperparathyroidism – jaw tumor) syndrome [18].

Extrarenal nephroblastomas are rare. They exist in two forms: nephroblastomas with the presence of additional tissue component and as an element of teratoma [19]. The latter form is usually located in retroperitoneal space, the inguinal region, ureters, ovaries, testes or in mediastinum [19-21]. Primary nephroblastomas of the uterus, colon, suprarenal gland and the wall of the chest were also described in
literature [22-25]. 10 to 15% of nephroblastomas metastasize – to lungs (57%), organs and lymph nodes of the abdominal cavity (14%), bones (14%) and liver (10%) [26].

Materials and methods

48 formalin-fixed and paraffin-embedded nephroblastoma tissue sections from the files of the Department of Pathology of the Age of Development and Department of Pathology, Konopnicka Memorial Hospital, Medical University of Lodz were selected for our study. From these paraffin blocks tissue samples about the thickness of 3-4 micrometers were prepared and stained with hematoxylin and eosin (HE). For the purpose of our study all of the previously diagnosed tumors (SIOP 93-01) were reclassified according to current criteria for this group (SIOP 2001 Classification of Renal Tumors of Childhood).

The estimation of the expression of investigated proteins was examined with computer image analysis system (Multi Scan Base v. 8.08 – Computer Scanning System, Ltd.). Selected histoclinical features: sex, presence of metastases or relapses, performed treatment, developmental defects, tumor volume, number of mitoses and survival time, histological type (grade or risk), histological subtype and stage of the disease were examined according to SIOP 93-01 and SIOP 2001 and were analyzed with the use of statistical package SYSTAT for Windows (Version 5.03, SYSTAT, Inc, Evaston, Illinois, USA, the license No.: DA021594) and the package „Survival“ (Version 1.0 Inc, Evaston, Illinois, USA, the license No.: DA061688). For measurable values we found the average and the median, standard deviation: 29.03; median: 42 months). Most of investigated nephroblastomas fell below 1000 cm$^3$ (the average: 828.8 cm$^3$; standard deviation: 1339.46 cm$^3$; median: 351.5 cm$^3$). The volume of tumors in investigated group is demonstrated in Fig. 1.

The time of patients’ observation ranged from 5 to 139 of months (the average: 35.12; standard deviation: 38.15; median 24 months). Ten children died because of neoplasmatic disease – survival time from 5 to 34 months (the average:13.5, standard deviation: 8.84; median: 10.5 months). In nine cases (18.75%) distant metastases were present, and recurrence in six cases (12.5%) within 7 to 9 of months of the end of the treatment (the average: 8; standard deviation: 1, median: 8 months). Among the cases diagnosed we found 15% of low grade, 76% of intermediate grade and 9% of high grade nephroblastomas according to SIOP 93-01. SIOP 2001 reclassification represented 9% of high risk, 43% of intermediate risk and 48% of low risk tumors. Diffuse anaplasia was found in three of all the examined nephroblastomas.

Histological risk and histological subtypes according to SIOP 2001

The strongest correlation was found between histological risk and high (over 20 in 10 hpf) number of mitoses under microscopic examination of tumor tissue sample, $p < 0.001$. High mitotic rate was observed exclusively in intermediate (7 cases) and high risk nephroblastomas (3 cases). A strong correlation was observed between histological risk and the death of the patients, both at $p = 0.005$. All fatalities appeared among patients with high (8 cases) and intermediate risk tumors (1 case). All the observed metastases appeared in high (6 cases) and intermediate risk tumors (2 cases). There were no statistically significant correlations between histological risk and the following parameters: age, sex, the presence of recurrence, developmental defects, tumor volume and stage of disease.

In the analysis of histological subtype according to SIOP 2001 statistically significant correlations were found with respect to: high number of mitoses, $p < 0.001$, death, $p = 0.014$ and stage according to SIOP 2001, $p = 0.02$. Mitoses at the rate of over 20 in 10 hpf were found exclusively in: epithelial (2 cases), stroma predominance (1 case) and blastemal
subtypes (4 cases). Most of fatalities occurred among patients with blastema Wilms’ tumors (66%). Blas
temal nephroblastomas predominantly represented highly advanced stages of disease (58%).

There was no statistically significant correlation between histological subtype according to SIOP 2001 and: neither age, sex, the presence of recurrence, developmental defects nor tumor volume, p > 0.05.

**Diffuse anaplasia**

Diffuse anaplasia strongly correlated with: stage (according to SIOP 93-01 and SIOP 2001), in both p = 0.001, fatalities, p = 0.006, and metastases, p = 0.008. There was no statistically significant correlation between diffuse anaplasia and: neither age, sex, developmental defects nor tumor volume, p > 0.05. Table 1 represents a summary of correlations of diffuse anaplasia in the examined group of patients.

**Histological grade and histological subtypes according to SIOP 93-01**

The strongest correlations were observed between histological grade and death, and histological grade and high number of mitoses, in both p = 0.002. All the fatalities occurred among patients with intermediate (7 cases) and high grade tumors (3 cases). A mitotic number of over 20 in 10 hpf was found exclusively among intermediate (6 cases) and high grade tumors (1 case). Histological grade correlated with recurrences as well, p = 0.01. All the recurrences appeared among patients with intermediate (4 cases) and high grade tumors (2 cases). There was a statistically significant correlation between histological grade and stage according to SIOP 93-01, p = 0.046, but there was no correlation with stage according to SIOP 2001, p > 0.05.

There was no statistically significant correlation between histological grade according to SIOP 93-01 and: neither age, sex, the presence of metastases, developmental abnormalities nor tumor volume, p > 0.05.

**Other features**

Developmental abnormalities correlated with metastases, p < 0.001, recurrences, p < 0.001 and high tumor volume (over 1000 cm³), p = 0.008. The main statistical data of analyzed features are displayed in Tables 2 and 3.

The log-rank and Kaplan-Meier images showed a shorter survival time among patients with:
- the highest stages of disease (III, IV, V), p = 0.021,
- relapses (8.74 vs. 22.4 months), p = 0.01,
- metastases (13.38 months), p < 0.001,
- diffuse anaplasia (9.50 vs. 29.52 months), p = 0.03.

Kaplan-Meier estimation of patients survival according to histological types is represented in Fig. 2 (SIOP 93-01) and Fig. 3. (SIOP 2001).

We also found that: age, sex, developmental defects, tumor volume, metastases and the number of mitoses did not affect survival time.

**Discussion**

The present therapy which encompasses preoperative chemotherapy, surgery and postoperative chemotherapy (with or without radiotherapy) allows for remission rate of approximately 60-70% in malignant neoplasms of childhood, and in some cases, e.g. Wilms’ tumor, of about 90% (6). In children malignancies the stage of disease is the basic prognostic factor. In our research we found correlations between the stage of the disease and the presence of metastases and fatalities. The time of survival of the patients with higher stages, metastases and recurrences was also shorter. However, the introduction of new classifications to the current treatment protocols is the proof that histological type or subtype of the tumor is also a prognostic factor of considerable importance [26-28]. Patients with nephroblastoma are treated in accordance with the stage of the disease and histological type of tumor [29]. In line with the SIOP protocols in all cases preoperative chemotherapy is performed, and only low-risk nephroblastomas and nephroblastoma in first stage of the disease do not demand the further post-surgical therapy. The retrospective analysis of the prognostic factors presented by the SIOP showed that in nephroblastoma group the histological type of tumor was the marker of greater importance than the stage of disease. In our research we compared histological types and subtypes of nephroblastoma according the two widely used SIOP classifications of renal tumors

Table 1. Summary of correlations of diffuse anaplasia in the examined group of patients

<table>
<thead>
<tr>
<th>Feature I</th>
<th>Feature II</th>
<th>P</th>
<th>Pearson ^2</th>
<th>Spearman Rho</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difuse anaplasia</td>
<td>death</td>
<td>0.006</td>
<td>14.561</td>
<td>0.352</td>
</tr>
<tr>
<td>Difuse anaplasia</td>
<td>metastases</td>
<td>0.008</td>
<td>13.906</td>
<td>0.329</td>
</tr>
<tr>
<td>Difuse anaplasia</td>
<td>recurrence</td>
<td>0.093</td>
<td>7.973</td>
<td>0.365</td>
</tr>
<tr>
<td>Difuse anaplasia</td>
<td>mitotic index</td>
<td>&lt; 0.001</td>
<td>48.455</td>
<td>0.568</td>
</tr>
<tr>
<td>Difuse anaplasia</td>
<td>stage according to SIOP 93-01</td>
<td>0.001</td>
<td>25.340</td>
<td>0.133</td>
</tr>
<tr>
<td>Difuse anaplasia</td>
<td>stage according to SIOP 2001</td>
<td>0.001</td>
<td>25.140</td>
<td>0.153</td>
</tr>
</tbody>
</table>
Table II. Results of statistical analysis of histoclinical features in nephroblastoma group in correlation with SIOP 93-01 (Feature II A) and SIOP 2001 (Feature II B) classifications

<table>
<thead>
<tr>
<th>FEATURE I</th>
<th>FEATURE II A</th>
<th>P</th>
<th>PEARSON χ²</th>
<th>SPEARMAN RHO</th>
<th>FEATURE II B</th>
<th>P</th>
<th>PEARSON χ²</th>
<th>SPEARMAN RHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td>grade</td>
<td>0.002</td>
<td>20.509</td>
<td>0.345</td>
<td>risk</td>
<td>0.005</td>
<td>18.474</td>
<td>0.380</td>
</tr>
<tr>
<td>Metastases</td>
<td>according</td>
<td>0.002</td>
<td>20.332</td>
<td>0.308</td>
<td>according</td>
<td>p&gt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitotic index</td>
<td>to SIOP 93-01</td>
<td>0.002</td>
<td>20.522</td>
<td>0.291</td>
<td>to SIOP 2001</td>
<td>&lt; 0.001</td>
<td>38.606</td>
<td>0.414</td>
</tr>
<tr>
<td>Recurrence</td>
<td>93-01</td>
<td>0.01</td>
<td>16.706</td>
<td>0.263</td>
<td>2001</td>
<td>p&gt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>histological</td>
<td>p &gt; 0.05</td>
<td></td>
<td></td>
<td>histological</td>
<td>0.014</td>
<td>28.044</td>
<td>0.393</td>
</tr>
<tr>
<td>Metastases</td>
<td>subtype-</td>
<td>p &gt; 0.05</td>
<td></td>
<td></td>
<td>subtype-</td>
<td>p&gt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitotic index</td>
<td>according</td>
<td>&lt; 0.001</td>
<td>41.829</td>
<td>0.378</td>
<td>according</td>
<td>&lt; 0.001</td>
<td>43.433</td>
<td>0.0</td>
</tr>
<tr>
<td>Recurrence</td>
<td>to SIOP 93-01</td>
<td>&gt; 0.05</td>
<td></td>
<td></td>
<td>to SIOP 2001</td>
<td>p&gt;0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table III. Statistically important correlations of developmental defects found in patients with nephroblastoma

<table>
<thead>
<tr>
<th>FEATURE I</th>
<th>FEATURE II</th>
<th>P</th>
<th>PEARSON χ²</th>
<th>SPEARMAN RHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Development defects</td>
<td>metastases</td>
<td>&lt; 0.001</td>
<td>24.254</td>
<td>0.301</td>
</tr>
<tr>
<td>recurrence</td>
<td>&lt; 0.001</td>
<td>27.496</td>
<td>0.348</td>
<td></td>
</tr>
<tr>
<td>tumor volume</td>
<td>0.008</td>
<td>17.489</td>
<td>—0.282</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Tumor volume in examined nephroblastoma group of tumors

Fig. 2. Kaplan-Meier estimation of patients survival in groups according to SIOP 93-01 histological types

Fig. 3. Kaplan-Meier estimation of patients survival in groups according to SIOP 2001 histological types

of childhood: SIOP 93-01 and SIOP 2001. Kaplan-Meier estimations made in compliance with both typologies indicated that histological types represented in SIOP 93-01 more distinctly separate the three nephroblastoma groups with different biological potential. In SIOP 2001 there was a clear separation of low grade nephroblastomas from other entities but all the remaining tumors (both intermediate and high grade) investigated in our study demonstrated mixed results of histoclinical investigation. Interesting results refer to an epithelial type of nephroblastoma currently categorized as intermediate risk tumor. In our estimations this subtype showed a mixture of results not easy to be interpreted. Division into previously used subtypes – well-dif-
ferentiated and poorly-differentiated epithelial nephroblastoma made the explanation clear. Well-differentiated nephroblastomas strictly represented the biology of today’s intermediate risk tumors. Poorly-differentiated nephroblastomas, however, shared some features with today’s high risk tumors.

The estimation of the value of numerous prognostic factors in children malignancies is needed. Results described in the literature are still under discussion. Most markers cannot be used in all of the groups of malignant tumors of childhood [30-32]. The assessment of tumor volume – an innovation introduced by SIOP 2001 – appeared an interesting feature which in our study correlated with the presence of developmental defects.

The final estimation of histoclinical features in nephroblastoma group still requires further research [33-35]. The value of tumor volume measurement, mitotic activity and other factors, e.g. developmental defects, give us the grounds to presume that despite many years of investigation there are some undiscovered and surprising areas in the biology of Wilms’ tumors.

In summary it is necessary to point out that the obtainment of satisfactory results of the treatment of children with neoplasmatic disease still depends on the creation and use of widely excepted protocols with the estimation of currently known and new prognostic factors.

References

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