A rare case of a Wilms tumor: case report

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Abstract

The nephroblastoma or Wilms tumor (WT) is the most common renal tumor in childhood, representing approximately 6–7% of all pediatric cancers, with a yearly incidence of 10 cases in one million children less than 15 years old, and continues to arouse interest by remarkable actual therapeutic successes, consecutive to the multidisciplinary approach. Its maximum incidence is around the age of 3–3.5 years old, having an equal frequency in males and females. We present the case of a child, aged three years and five months, who was diagnosed with WT (nephroblastoma) with triphasic pattern, stage II tumor, and admitted to the Department of Oncopediatry for chemotherapeutic treatment and clinico-biological investigations.

Keywords: Wilms tumor, nephroblastoma, nephroureterectomy, triphasic pattern, immunohistochemistry.

Introduction

Wilms tumor (WT), also named nephroblastoma, currently ranks first amongst malignant tumor affecting the kidneys in children [1]. It is not usual to be found in both kidneys at the same time, mostly being unilateral in native organs [1–3]. Although it is considered a rare disease, it is the most frequent solid tumor in childhood, according to specialty literature statistics, representing about 5% of all cancers that occur in children, with an increased rate of curability (90%), due to a combined surgical and oncological treatment. The disease affects approximately 1/10 000 children [4, 5], eight in a million children less than 14 years old, respectively [5]. Children between one and four years of age are the most affected category (65% of all cases).

Described by Wilms, in 1899, the nephroblastoma is a “bizarre” malignant neoplasia with triphasic development from the embryonal, pluripotent, precursory kidney cells: undifferentiated metanephric blastema, fibroblast-like and epithelial stromal elements. They are usually sporadic, familial cases, representing only 1% of records. In the majority of cases, WT occurred between six months and five years of age; the gender distribution is equal among boys and girls. Anaplastic forms are present in 5% of cases [4–6].

While some WTs may display a primary cellular type, most occurrences contain a mixture of three different cellular types – undifferentiated blastemal cells, epithelial cells with different degrees of differentiation, as well as various stromal elements. Pluripotential mesenchymal cells of the kidney seem to be the root element for these components, as they fail to undergo normal differentiation [7]. Rhabdomyogenesisis, translated by striated and smooth muscle cellular elements, along with chondrocytes, osteocytes and sometimes adipocytes make up the ectopic mesenchymal elements that are usually found in the triphasic WT [8]. These unusual elements are accompanied by almost normal structures that can be found during nephrogenesis – both epithelial and blastemal. This is why WTs are usually considered mesenchymal, having ectopic differentiation; the cell types being reminiscent of the progenitor mesenchymal stem cells that can differentiate into connective tissue elements, bone, adipocyte or muscle [7–9].

Aim

We present a rare case of a WT in a young female patient that presented with non-specific symptoms and posed diagnostic challenges.

Case presentation

We present the case of a girl, aged three years and five months at the time of admission, diagnosed with WT with triphasic pattern, stage II tumor, and admitted to 2nd Pediatric Clinic, Department of Oncopediatry, for chemotherapeutic treatment.

She was the second child of young and healthy parents, born on term, birth weight of 3950 g, with no birth suffering, breast-fed until one year and four months, with food diversification from the age of four months. Normal physical and psychomotor development, according to age stages. At two years, she suffered a trauma on the proximal extremity of the right calf.

The disease had its onset seven months before the Hospital admission (early 2014), the main symptoms being an increase of the abdomen volume, perspiration mainly at night, and weight loss. Subsequently, she had hematuria and was admitted to a County Hospital, with the diagnosis
of urinary infection. The abdominal ultrasound revealed a left renal tumor, the patient being transferred to the Pediatric Surgical Clinic of the Emergency County Hospital of Craiova, Romania. The weight upon admission was 12 kg, 91 cm height, 0.52 m² body surface, normal development with slightly pale teguments, supple abdomen, normal transit and spontaneous urine emissions. Blood work showed a hemoglobin (Hb) of 10.2 g%, 3 820 000 erythrocytes/mm³, 4300 leukocytes/mm³ (42% lymphocytes), thrombocyte count 293 000/mm³, anisocytosis, anisochromia. We found values for glutamate–pyruvate transaminase (GPT) 37 U/L, creatinine 0.45 mg%, urea 24 mg%, total proteins 6 g%, serum iron 17 µg/dL, ionic calcium 3.93 mg%, magnesium 2.19 mg% and phosphorus 4.4 mg/dL. Urine – rare epithelial cells, leukocytes and erythrocytes.

We performed an abdominal and pelvic computed tomography (CT) scan, revealing a tumor mass on the left side of 10.7×8.9 cm. The formation had tissued density, with central necrosis, incorporating the left renal artery. The higher pole of the formation was tangent to the spleen and stomach, while the lower pole was in the small basin. The CT scan also showed peritumoral fluid. The right kidney was normal, with retained hepatic and splenic integrity. The pancreas was pushed higher because of the abdominal formation. No sign of urinary bladder alteration or suspicious bone modifications. The pulmonary CT scan revealed normal relations.

We proceeded to surgical intervention – left nephroureterectomy, with no immediate complications.

A histopathological examination was performed.

**Histopathological technique**

The biological material consisted of the nephrectomy piece, which was fixed in 10% buffered formalin and subsequently processed by classical histopathological technique, which involved inclusion in paraffin, 3–5 µm sectioning and Hematoxylin–Eosin (HE) staining (Bio kit, Optica, Romania).

For the immunohistochemical (IHC) analysis, 4 µm-thick serial sections were performed, which were applied on poly-L-lysine slides and included at thermostat, at 37°C, for six hours. Endogenous peroxidase blocking and non-specific blocking of antigenic sites were performed for each reaction. Monoclonal antibodies produced in mouse (anti-human mouse) were used.

The antibody panel used, clone, dilution, antigen display and external positive controls are shown in Table 1.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Dilution</th>
<th>Antigen demasking</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
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<td>EDTA buffer, pH 9</td>
<td>kidney</td>
</tr>
<tr>
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<td>1:50</td>
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<td>colon</td>
</tr>
<tr>
<td>CK</td>
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<td>EDTA buffer, pH 9</td>
<td>kidney</td>
</tr>
<tr>
<td>α-SMA</td>
<td>1A4</td>
<td>1:50</td>
<td>Citrate buffer, pH 6</td>
<td>colon</td>
</tr>
<tr>
<td>Ki67</td>
<td>MIB-1</td>
<td>1:50</td>
<td>Citrate buffer, pH 6</td>
<td>mammary carcinoma</td>
</tr>
</tbody>
</table>

WT: Wilms tumor; CK: Cytokeratin; α-SMA: Alpha-smooth muscle actin; EDTA: Ethylenediaminetetraacetic acid.

The work system used was based on polymer amplification (Novolink Polymer DS, code RE7150-CE, Leica Biosystems), and the reaction visualization was obtained with 3,3’-Diaminobenzidine (DAB, code RE7190-CE, Novocrosta, Leica Biosystems) bookmarks.

IHC reactions were evaluated as localization, intensity and percentage of marked cells. The intensity of the markings has been graded as increased, moderate or weak. The areas at which the measurements were made were identified at a 10× microscopic target, and the cells were counted at a 40× microscope magnification. Subsequently, the number of positive cells per 100 cells count was reported. For Ki67, the proliferation index was calculated, and for the other labels, the reactions were considered diffuse (over 50% of marked cells) or focal (less than 50% of marked cells).

**Histopathological analysis**

The blastic component consisted of small cell groups with low cytoplasm, hyperchromic nuclei and mitotic activity arranged in islands, sometimes anastomosed, clearly delineated by the surrounding stroma, sometimes with the presence of epithelial tubular structures within the islands.

The epithelial component showed tubular delivery with the presence of small tubules of cuboidal-columnar cells with oval nuclei.

The mesenchymal component consisted of a predominantly fusiform cell population disposed in a weak basophilic matrix (Figure 1).

The renal parenchyma was atrophied by compression from the adjacent tumor (Figure 2). The blastem component predominated in this WT case (Figures 3 and 4).

**IHC analysis**

The WT1 immunoassay was identified at the nucleus of the blastic tumor cells, the epithelial and mesenchymal components being negative. The marking was diffuse with varying intensity (Figures 5 and 6).

Immunoreactivity of vimentin was identified cytoplasmatically at the mesenchymal component, the reaction being a diffuse high intensity. Immunoreaction was present at the level of blastic and epithelial components, which suggested the epithelial–mesenchymal transition and blastic–mesenchymal transition capacity of these components (Figures 7 and 8).

Immunoreactivity of cytokeratin (CK) AE1/AE3 was only identified in the cytoplasm of epithelial tumor cells, the marker being diffuse and intense, and the blastic and epithelial components were negative (Figures 9 and 10).

The alpha-smooth muscle actin (α-SMA) marker was observed only in the cytoplasm of the mesenchymal elements and the tumor mesenchymal component, the mark being a diffuse, intense one (Figures 11 and 12).

The mean Ki67 proliferation index was 26% for the blast-body component, and less than 10% for both epithelial and mesenchymal components, which may indicate the blastic component as the main tumor proliferative compartment (Figures 13 and 14).
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Figure 1 – Blastic component, both epithelial and mesenchymal, pertaining to the tumor (HE staining, ×100).

Figure 2 – Atrophic renal parenchyma, adjacent to the tumor, showing a smaller cellular component, with cytoplasmic alterations (HE staining, ×100).

Figure 3 – Predominant blastic component within the tumor, hyperchromic nuclei and less cytoplasmic material (HE staining, ×100).

Figure 4 – A detail of the blastic tumor component, showing the islet pattern of the cells (HE staining, ×200).

Figure 5 – Nuclei of blastic tumor cells showed an affinity for the WT1 immunomarker, with various degrees of intensity (Anti-WT1 antibody immunostaining, ×100). WT1: Wilms tumor 1.

Figure 6 – We found most epithelial and mesenchymal components negative for WT1, as opposed to cell nuclei within the blastic region (Anti-WT1 antibody immunostaining, ×100). WT1: Wilms tumor 1.
Figure 7 – We used vimentin in order to determine the mesenchymal component, as the cellular cytoplasm within these sectors was intensely positive (Anti-vimentin antibody immunostaining, ×100).

Figure 8 – As opposed to only showing vimentin positivity within the mesenchymal sector, we encountered vimentin activity at the blastic and epithelial level, suggesting transitional behavior (Anti-vimentin antibody immunostaining, ×100).

Figure 9 – Negative CK AE1/AE3 nuclear staining within the blastic and epithelial components of the tumor, with intense activity within epithelial cells (Anti-CK AE1/AE3 antibody immunostaining, ×100). CK: Cytokeratin.

Figure 10 – Positive cytoplasm of cells pertaining to the epithelial components within the tumor (Anti-CK AE1/AE3 antibody immunostaining, ×100). CK: Cytokeratin.

Figure 11 – Intense positivity for α-SMA within the cytoplasm of mesenchymal cells (Anti-α-SMA antibody immunostaining, ×100). α-SMA: Alpha-smooth muscle actin.

Figure 12 – A detail, showing the specificity of the immunostaining for mesenchymal elements, with diffuse but intense disposition (Anti-α-SMA antibody immunostaining, ×200). α-SMA: Alpha-smooth muscle actin.
Case outcome and follow-up data

She was transferred to the 2nd Pediatric Clinic, Department of Oncopediatry, Emergency County Hospital of Craiova, where, after performing the biochemical investigations [hemogram, erythrocyte sedimentation rate (ESR), fibrinogen, urea, amylasemia, uric acid, glycemia, transaminase, electrophoresis of serum proteins, bilirubin, calcium, serum iron test, phosphatemia, serum phosphatases, coagulation tests, serum immunogram] and the medullogram, the cytostatic treatment started.

The patient continued cytostatic treatment in our Clinic until late 2014.

Afterwards, the child continued cytostatic treatment at another Hospital between 2015 and 2016, with good overall status and prognosis. Treatment was continued abroad, with positive outcome at the time of writing.

Discussions

According to some studies, approximately 650 new cases of WT are reported in the United States each year; approximately 5% present as synchronous bilateral tumors [10, 11], while only 2–3% may evolve a metachronous tumor [10–12]. The Asian population has a lower incidence of WT [13, 14]. This is most likely a genetic factor, as this lower incidence is found even in migrated cohorts and results from variations in molecular pathogenesis of precursor lesions for WT, such as nephrogenic rests [8, 14]. The WT1 gene is most likely linked to renal tumors in children, WT being a product of the inactivation of both alleles of this respective gene, found on the 11p13 chromosome [14–18]. A zinc finger related transcription factor that is encoded by the WT1 gene plays a significant role in cell growth and differentiation. Progenitor cells found in some tissues, mainly of the mesothelium, uterus, kidneys or gonads have higher expression rates for this factor [16–18]. This was proven in a study on WT1 knockout mice that exhibited deficiencies of the genitourinary system and having a short lifespan [19].

Beckwith & Palmer [20] considered WT as an embryonal renal tumor in which blast, stromal and epithelial-type cells are present. According to Machin [21], the blast elements or the nephroblastomatosis would constitute the WT precursors. Pochedly [22] stated that oncogenesis would take place through hamartomas, which produce blast tumors and, following crowding and unification, would form a WT, which contains in various proportions the three cellular types. Several studies have shown that WT developed from blast elements, while the mesoblastic nephroma developed from stromal type cells, and the carcinomatous renal cells would result from epithelial cells [23–25].

Literature notes a series of congenital malformations that are most likely related to WT; finding them represents an important diagnosis argument in the case of a patient with abdominal tumor [8, 9]. The most significant characteristic is the aniridia (congenital absence of the iris). Other associated malformations are hemihypertrophy, Beckwith–Wiedemann syndrome (gigantism, visceromegaly, and neonatal hypoglycemia), hypospadias, cryptorchidism and renopyeloureteral duplications [9–11]. Clinical aspects may include delayed growth, bad state, the child having the tendency to stop playing, presenting sleepiness, apathy, and sometimes fever. Other symptoms that could set the diagnosis of renal tumor may include the presence of an abdominal tumor, which can be an accidental discovery of the family in a child with a good general state [9–11]. The tumor is often voluminous, previously developed, with a firm, smooth, soft, slightly mobile, and unpainful consistency [1, 4]. It rapidly grows in volume, which is noticeable from one examination to another, in a short interval of time. Sometimes, the child presents stomachaches, suggesting an acute abdomen; this pain could represent a tumor rupture. The presence of hematuria (in 20% of the cases) and high blood pressure (in 25% of the cases) can also be noted [11, 26]. The presence of a left varicocele, which can be explained through the fact that the tumor thrombus initially invades the left renal vein and causes the rupture of the venous return through the left spermatic vein, is also symptomatic [1–4].

Although rarer, bilateral WT is often associated with genetic syndromes that predispose to renal disease, thus having an early onset [1, 19]. As a large percentage of the total renal parenchyma is rendered inert by a bilateral presentation, renal insufficiency is the most significant co-morbidity [19].
Due to improved diagnostics and more advanced procedures, recent years saw a dramatic increase in survivability, up to 90% of patients with WT compared to just 30% some decades ago [27, 28]. Curative protocols now include both surgery and irradiation or chemotherapy, their efficiency being proven through large-scale randomized multicenter trials. International cooperative trials have been developed by the societies of pediatric oncology in both Europe and the United States, studying therapy options and outcomes in the last five decades [1–3, 27, 28].

Conclusions

Early diagnosis of the WT is essential, as symptoms may vary and the lack of annametic data may sometimes present as a problem for the practitioner. Histology and immunohistochemistry studies are necessary in order to determine the three components of the tumor and can give way to targeted therapies in the near future.

Conflict of interests

The authors declare that they have no conflict of interests.

Authors’ contribution

Cristina Elena Singer and Laura Daniela Marină contributed equally to the paper and share first authorship.

References


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