Prediction of histopathological local staging by radiological findings and differential diagnosis overview in children with nephroblastoma

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Abstract

Background: Nephroblastoma is the most common renal malignancy in children kidney. They are highly heterogeneous tumors with challenging imagistic and histopathological (HP) differential diagnosis. Imaging is critical for understanding local anatomy, staging and for planning surgical approach. Purpose: To determine whether HP staging can be successfully predicted by the imagistic staging using computed tomography. Also, we find it important to make a brief review of the imagistic, HP and immunohistochemical differential diagnosis of nephroblastoma, considering that a correct diagnosis is essential for an appropriate therapeutic strategy in all stages. Patients, Materials and Methods: We present a retrospective study of the medical dossiers of 22 patients that underwent surgery at our Center between 2014 and 2020. We provided descriptive data and compared imagistic and HP staging using a Mann–Whitney U-test. An up-to-date literature review was also done. Results: We found that imagistic staging tends to under- or over-stage at similar rates and that the difference between the two staging systems is statistically significant. Immunohistochemistry is necessary for establishing the correct diagnosis, especially in cases with one predominant HP pattern. Conclusions: HP and imagistic staging are not yet sufficiently similar for successfully predict the former via imagistic means.

Keywords: nephroblastoma, staging, histopathology, immunohistochemistry, computed tomography.

Introduction

Nephroblastoma is a malignant embryonic tumor originating from renal precursor cells, consisting of an inconstant percentage of embryonal renal components: blastema, epithelium and stroma [1, 2]. Wilms tumor (WT) is the most frequent renal tumor in children and represents about 6% of all malignancies in childhood [2]. Consecutive clinical trials organized by the National Wilms Tumor Study Group (NWTSG), which was superseded by the Children’s Oncology Group (COG) in 2002, the International Society of Pediatric Oncology (Société Internationale d’Oncologie Pédiatrique – SIOP) and other national study groups established an overall survival rate over 90% [3, 4]. The difference between the therapeutic approaches in the NWTSG/COG and SIOP protocols has repercussion for the imaging method used for diagnosis. In the NWTSG/COG protocol, chemotherapy and radiotherapy follows surgical treatment, so imaging methods are used to add accurate anatomical description for staging and devising a personalized surgical strategy [4, 5]. Echography is the first chosen imaging method, confirming the existence of a renal tumor without ionizing radiation and allows an initial evaluation of the other kidney [6, 7]. However, magnetic resonance imaging (MRI) or computed tomography (CT) are necessary for gaining all information for diagnosis and staging [6]. While imaging [ultrasonography (US), CT, and MRI] can predict up to 95% of nephroblastomas, they cannot predict the histopathological (HP) subtypes and therefore HP examination is mandatory [8]. Accurate HP description and stadialization of these tumors are essential since their treatment and outcomes varies [9].

Aim

The aim of our study was to determine whether HP staging can be successfully predicted by the imagistic staging using CT.

Patients, Materials and Methods

Patients

We performed a retrospective study using our institutional database to identify patients who underwent surgical treatment for nephroblastoma at our center during a 6-year period, between September 2014 and September 2020. We identified a total of 33 patients initially diagnosed with renal tumors presenting nephroblastoma-like characteristics on CT scans. From this group, we excluded five patients who underwent surgical treatment at a different Center, two patients who had a different HP diagnosis [clear cell renal carcinoma and intrarenal neuroblastoma (NB)], one patient who was treated following SIOP protocol and three patients who were lost to follow-up. A total of 22 patients histopathologically confirmed with nephroblastoma were left to be included in the analysis. Patient demographics, including age, gender, urban or rural environment, associated syndromes, presenting signs and symptoms, oncological
and surgical management were determined from a review of each patient’s medical record.

**Methods**

Thoraco-abdomino-pelvic CT studies were used for staging in all cases. Abdominal US was also used in all cases as the initial diagnostic method. Postoperative local staging in all cases. Abdominal US was also used in all of each patient’s medical record.

**Results**

We reviewed the records of 22 patients who underwent surgery for nephroblastomas at our Center. The median age at diagnosis was three years ranging from 0 to 11 years. The male to female ratio was 1:1. There were 16 (73%) patients from rural areas and six (27%) patients from urban areas. Associated anomalies were found in only one patient who had Beckwith–Wiedemann syndrome. There were 15 patients that presented with abdominal pain, 14 with palpable abdominal mass, nine with weight loss and six with hematuria. Less frequent manifestations were fever, loss of appetite, vomiting and acute urine retention (Table 2).

**Data analysis**

Descriptive data was analyzed using Microsoft Excel. Staging results were statistically analyzed using Statistical Package for the Social Sciences (SPSS) software. All patients were categorized into five classes according to their neoplastic staging at the time of diagnosis and to the HP local staging. The two staging distributions were compared using a Mann–Whitney U-test. A level of significance \( \alpha = 0.05 \) was used.

**Radiological staging**

Only one patient (4.5%) in our study group had bilateral nephroblastoma. Vascular invasion was objectified in four (18.1%) cases, renal pelvis or ureteral invasion in five (22.7%) cases, capsular effraction in five (22.7%) cases and adjacent organ invasion in four (18.1%) cases.
lymphadenopathies in 10 (45.4%) cases and metastases in five (22.7%) cases. Most tumors were classified as stage III (40.9%), followed by stage II (27.2%) and stage IV (55.7%) based on imaging studies. Only one patient (4.5%) was classified as stage I (Figure 1).

**HP staging and IHC**

Renal tumors from 22 children treated at our Institution between 2014 and 2020 were histopathologically studied. Only one case (4.5%) was biopsied prior to chemotherapy. In all other cases the entire tumor was received as a resection specimen. Neoadjuvant chemotherapy was administered in eight (34.7%) cases, for stage III unresectable tumors, stage IV and V tumors. Post-chemotherapy tumor necrosis was described in only one case (4.5%). A predominant HP pattern (Figure 2, a–d) was found in four (18.1%) tumors. Three (13.6%) tumors had a biphasic pattern and the majority, 14 (63.6%), had a triphasic pattern (Figure 3, a–e). In all monophasic tumors, blastemal cells were predominant. All biphasic tumors had epithelial and blastemal elements. Only one tumor had focal anaplasia. Based on predominant cell types, tumors were classified into low, intermediate, or high risk. Most patients had an intermediate risk, 13 (59%), while low risk was attributed to only three (6.6%) patients and high risk to six (27.2%) patients (Figure 4).

**Figure 1** – Frontal and transversal computed tomography (CT) sections of different nephroblastoma stages.

**Figure 2** – Blastemal, epithelial and stromal component: (a) Mesenchymal component represented by loose connective tissue and small vessels; (b) Blastemal component formed by small ovoid cells with reduced eosinophilic cytoplasm and hyperchromatic nuclei showing high mitotic rate; (c) Epithelial component forming closely packed rosettes, resembling neuroectodermal tissue; (d) Biphasic pattern in nephroblastoma showing epithelial component forming small tubules and surrounded by mesenchymal component. Hematoxylin–Eosin (HE) staining: (a and d) ×100; (c) ×200; (b) ×400.
Figure 3 – Biphasic and triphasic pattern of nephroblastoma: (a) Triphasic pattern in nephroblastoma with micronodules of blastema (bottom left), epithelial component forming tubules and immature glomeruli, connected by connective tissue with variable fibrotic stroma; (b) Triphasic pattern in nephroblastoma depicting islets of blastema (bottom field), small tubules (central) embedded in a loose connective tissue; (c) Biphasic pattern depicting well-formed tubules and incomplete glomeruli in the epithelial component (center), enclosed by the blastemal tissue; (d) Triphasic nephroblastoma with intermingled elements; (e) Triphasic nephroblastoma illustrating blastema (right) mixed with tubules and immature glomeruli and loose mesenchyme (left). HE staining: (a–c) ×100; (d) ×50; (e) ×200.

Figure 4 – Histopathological (HP) risk stratification.

Immunohistochemistry was performed in 12 (55%) cases (Figure 5, a–d). Ki67 proliferation index was calculated. It ranged from 20% to 90%, with the highest value in stage II – 70%, in stage III – 75%, and in stage IV – 90%. A panel of markers were used to establish the differential diagnosis according to the predominant HP pattern. In our analysis, all monophasic nephroblastoma had a blastemal predominant pattern, and in all cases, immunohistochemistry markers were used to obtain a certain diagnosis, including WT1, pan-cytokeratin (CK) AE1/AE3, desmin and paired-box 8 (PAX8). In all cases, immunohistochemistry confirmed the diagnosis of monophasic nephroblastoma.

Various elements were examined to establish correct staging of nephroblastomas: renal capsule involvement, ureteral extension, vascular invasion, lymph node involvement and surgical margins status (Figure 6, a–d). Capsular effraction was seen in 10 (45.4%) patients, renal pelvis or
ureteral extension in six (27.3%) patients, microvascular
invasion in 11 (50%) patients and lymph node involvement
in 10 (45.4%) patients. Most tumors, 19 (86.3%), had clear
resection margins, while in three (13.7%) cases complete
excision was not achieved. Most of the tumors were
classified as stage III (40.9%), followed by stage II and stage
IV (27.2% each). Two children had stage I nephroblastoma
(9%). Only one patient had bilateral nephroblastoma—
stage V (4.5%) (Table 3).

Table 3 – Imagistic and HP staging criteria

| Criteria                  | CT (% of cases) | Histopathology (% of cases) |
|---------------------------|-----------------|-----------------------------|
| Vascular invasion         | 18.1%           | 50%                         |
| Basinetal/ureteral invasion| 22.7%           | 27.3%                       |
| Capsular effraction       | 22.7%           | 47.5%                       |
| Lymph node invasion       | 45.4%           | 45.4%                       |

CT: Computed tomography; HP: Histopathological.

Radiological and HP staging correlation

In a review of the records of all children confirmed with
nephroblastoma that underwent surgery at our Center, we
found 22 patients that were classified into five categories
according to their imagistic and HP stage. We found one
patient with stage I, six with stage II, eight with stage III,
four with stage IV and one with stage V (Figure 7).

We found four patients without correlation between
imagistic and HP staging. Two of these patients had an
imagistic stage II but HP stage III, one had an imagistic
stage III, but HP stage II and one had imagistic stage III
and a HP stage I (Table 4).

Table 4 – HP staging compared to CT staging (I–V)

| HP staging | CT I (n) | CT II (n) | CT III (n) | CT IV (n) | CT V (n) | Total |
|------------|----------|-----------|------------|-----------|----------|-------|
| I          | 1        | 0         | 1          | 0         | 0        | 2     |
| II         | 0        | 4         | 1          | 0         | 0        | 5     |
| III        | 0        | 2         | 7          | 0         | 0        | 9     |
| IV         | 0        | 0         | 0          | 5         | 0        | 5     |
| V          | 0        | 0         | 0          | 0         | 1        | 1     |
| Total      | 1        | 6         | 9          | 5         | 1        | 22    |

CT: Computed tomography; HP: Histopathological; n: No. of cases.
Figure 6 – Histopathological aspect of lymphovascular, sinus and capsular invasion: (a) Necrotic area showing cellular and nuclear debris in an aggressive nephroblastoma, blastemal-dominant; (b) Vascular invasion showing blastemal embolus; (c) Tumor invasion into the fibrous capsule, below the upper pole (adrenal tissue-upper field); (d) Vascular invasion showing blastemal embolus. HE staining: (a, b and d) ×100; (c) ×25.

Figure 7 – Imagistic and histopathological (HP) stage correlation.

Discussion

Imagistic techniques

Imagistic evaluation for renal tumors should begin with abdominal US, that can establish if the tumor is intrarenal or extrarenal and if its predominant component is dense or cystic [7, 10]. We performed abdominal US in all cases as the initial imagistic method. On US, nephroblastoma usually appears as a large intrarenal mass that shows heterogeneous echotexture, well-defined margins, delimited by a pseudocapsule [5, 11]. Nephroblastomas can have extensive hypoechoic regions representing central necrosis or cyst development. Hyperechoic regions can be areas of fat, calcification, or hemorrhage. It can also appear as a solid round mass, less frequent [4, 5]. US is helpful for the establishment of caval permeability and inferior vena cava tumor thrombus [12, 13]. We identified one case of nephroblastoma with intravenous extension visible on US, in a two-year-old child. Because US is operator dependent and regarding the limited field of vision, supplementary imaging methods for extended characterization and precise staging are necessary [7].

In a few cases, nephroblastoma can be predominantly cystic, called cystic partially differentiated nephroblastoma, and can be hard to differentiate from other cystic renal tumors, benign and malignant [14, 15, 16]. We identified one case of a 2-year-old boy who presented for fever and vomiting. In imaging studies, two round, well defined lesions with fluid density were identified in the right kidney. The initial clinic and imagistic diagnosis were renal abscesses. Intraoperatively two well defined, encapsulated tumors that had an elastic consistency and white color were identified. Lesion excision was performed. HP examination established
the diagnosis of biphasic nephroblastoma. Postoperative chemotherapy was administered.

CT of the abdomen with administration of intravenous iodinated contrast is also used for diagnosis and evaluation of nephroblastomas [14, 17, 18]. We performed CT scans as the main imagistic method for preoperative staging in all patients. On CT, nephroblastoma is described as a heterogeneous soft-tissue density tumor with less enhancement compared to the adjacent renal tissue [19]. Nephroblastomas often contain hypodense regions because of necrosis, hemorrhage or cysts, but fat and calcifications can be seen as well [20, 21]. More CT aspects can comprise of infiltration or distortion of the pyleocalceal system, vascular extension or lymph node involvement [17]. CT allows appropriate staging of the disorder and identifies the existence of nephrogenic rests (NR) in the renal tissue [13, 15]. Abdominal CT can identify the existence of tumor rupture, representing an important risk factor for recurrence of the disease [2]. Less characteristic imagistic aspects are perirenal bleeding that can exist without identifying a tumor rupture, retroperitoneal liquid and intra-abdominal peritoneal effusion [15].

Initially, the differential diagnosis of nephroblastoma is done with extrarenal intra-abdominal tumors. When the renal origin of the tumor is settled, other disorders to be included in the differential diagnosis of nephroblastoma are congenital mesoblastic nephroma, clear cell sarcoma of the kidney (CCSK), malignant rhabdoid tumor of the kidney and renal cell carcinoma (RCC), also known as non-WTs. Non-WTs are frequently identical with nephroblastoma on imaging studies [15, 21].

Congenital mesoblastic nephromas are histopathologically divided into classical and cellular subtypes. On CT scans, the classical subtype looks like a homogeneous solid tumor and the cellular form is heterogeneous with regions of necrosis, hemorrhage, and cystic degeneration [22].

RCC appears smaller on CT compared to nephroblastoma, enlarges progressively and may penetrate neighboring structures altering normal kidney architecture [23]. In comparison with nephroblastoma, RCC is frequently bilateral, and calcifications are often found [16]. We identified one case of an 8-year-old girl who presented with a massive, palpable abdominal tumor. Abdominal US was performed, identifying a right renal tumor. Abdominal MRI was performed for supplementary characterization of the tumor and identified a large (117/115/151 mm) renal tumor, with hyperintensity on T2-weighted images, compatible with renal nephroblastoma. Preoperative chemotherapy was administered, according to SIOP-2001 protocols for nephroblastoma, followed by nephrectomy. HP studies in association with IHC analysis established the diagnosis of clear cell renal carcinoma with alveolar soft part sarcoma locus (ASPL)–transcription factor binding to immunoglobulin heavy constant mu (IGHM) enhancer 3 (TFE3) translocation. Surgical excision was followed by adjuvant chemotherapy and radiotherapy.

**HP and IHC overview**

Typically, nephroblastomas comprise of three HP components, which are the basis for the differential diagnosis: mesenchymal elements resembling primitive fetal mesenchyme (blastema), epithelial elements that recall fetal renal tubules and glomeruli and blastomatous elements representing clusters of blast cells that are involved in the coinage of the term nephroblastoma [9, 24]. The amplitude and the degree of maturation of these elements vary considerably, resulting in a distinguishable HP appearance of each tumor [25].

The diagnosis is usually not problematic in triphasic or even biphasic nephroblastoma, but if only one element is found, particularly in a small biopsy sample, the differential diagnosis can be difficult [26]. In our study, we identified four cases of monophasic pattern, all of them having blastemal predominance and three cases of biphasic pattern. IHC is helpful in reaching a definitive diagnosis in most cases. Different IHC panels are indicated based on the HP predominant pattern (Table 5) [26].

| Patterns | Recommended panel | Additional panel |
|----------|------------------|-----------------|
| **Epithelial pattern** | | |
| WT1       | CK7              | PAX8            |
| Pan-keratin | TFE3*            | BCOR*           |
| Melanocytic markers | TFEB*            | Cathepsin K  |
| AMACR      | BRAF V600E*      | ALK             |
| Cyclin D1  |                  | Cyclin D1      |
| **Stromal pattern** | | |
| WT1       | BCL-2            | INI1            |
| CD34      | NGFR*            | CD99            |
| Cyclin D1 | BCOR*            | BRAF V600E*    |
| **Blastemal pattern** | | |
| WT1       | NE markers       | CD56            |
| INI1      | Cyclin D1        | CD99            |
| CKs       | NB84*            | CD45            |
| Desmin    | NGFR*            | |

Ki67, a tumor proliferation marker with prognostic significance, is evaluated in nephroblastomas. High Ki67 proliferation index is associated with more aggressive clinical behavior and is proved to be an important determinant of distant metastasis and tumor related deaths [27].

**Blastema**

Blastema is the least differentiated and hypothesized the most malignant element [25]. It is composed of small blue cells with overlapping nuclei and brisk mitotic activity. Primitive tubular epithelial elements can be found in the center of blastemal nodules and morphologically mimic NB-like areas with pseudorosettes [28].

Pure blastemal type nephroblastoma may be difficult to distinguish from other embryonal small round blue cell tumors, including NB, malignant rhabdoid tumor of the kidney, primitive neuroectodermal tumor, Ewing sarcoma (EWS), desmoplastic small round cell tumor (DSRCT) and lymphoma [24, 26]. To reach the correct diagnosis in cases of difficult differential diagnosis it is essential to use...
immunohistochemistry and molecular biology in search for distinctive elements (Table 6) [29]. The blastemal elements of nephroblastoma present nuclear WT1 positivity in 80% of cases [30].

**Table 6 – IHC characteristics of the most common renal tumors with a blastemal pattern**

| Blastemal pattern | WT1(n) | INI1 | NGFR | Keratin | CD99 | NE | Desmin | PAX8 | NB84 | Cyclin D1 |
|-------------------|--------|------|------|---------|------|----|--------|------|------|----------|
| WT                | +      | ++   | -    | +/-     | +/-  | NA | +/-    | -    | ++   | -        |
| MRTK              | -      | -    | +    | +/-     | +/-  | NA | +/-    | -    | ++   | +        |
| NB                | -      | ++   | +    | -       | ++   | NA | ++     | -    | ++   | +        |
| DSRCT             | +      | ++   | -    | +/-     | --   | NA | ++     | -    | ++   | -/+      |
| EWS               | -      | ++   | -    | +/-     | --   | NA | +/-    | -    | +/-  | ++       |

CD99: Cluster of differentiation 99; DSRCT: Desmoplastic small round cell tumor; EWS: Ewing sarcoma; IHC: Immunohistochemical; INI1: Integrase interactor 1; MRTK: Malignant rhabdoid tumor of the kidney; n: Nuclear; NA: Not available; NB: Neuroblastoma; NE: Neuroendocrine; NGFR: Nerve growth factor receptor; PAX8: Paired-box 8; WT: Wilms tumor. Scoring: ++ >95% positive cases; + 76–95% positive cases; +/- 51–75% positive cases; - 5–25% positive cases; -- <5% positive cases.

DSRCTs share several IHC characteristics with blastemal-type nephroblastoma, but are rare and the diagnosis should be established only if genetic tests identify EWS–WT1/t(11;22)(q13;q12) translocation [17, 24]. DSRCT are WT1-positive but only with the C-terminal clone of WT1, and nephroblastomas are positive with both the N-terminal and C-terminal clone. DSRCT also show CK and desmin positivity. PAX8 is negative in DSRCT [29]. In our study group, we identified one case of a six-year-old boy radiologically diagnosed with three intra-abdominal heterogeneous masses, the larger one localized in the left hemiabdomen (98/90/128 mm) and the other two in the pelvic region and an intrathoracic tumor with similar characteristics. The initial radiological suspicion was of NB. A biopsy was performed, and HP examination identified a malignant DSRCT. HP and IHC examination of the entire tumor after nephrectomy established the diagnosis of biphasic nephroblastoma with 95% blastemal cells and 5% epithelial component.

**Table 7 – IHC characteristics of the most common renal tumors with an epithelial pattern**

| Epithelial pattern | WT1 | AMACR | Pan-keratin | CK7 | TFE3 | TFEB | Melanocytic markers | BRAF V600E |
|-------------------|-----|-------|-------------|-----|------|------|---------------------|-----------|
| WT                | ++  | --    | +/-         | --  | --   | --   | --                  | --        |
| NR                | --  | NA    | +           | +/- | NA   | NA   | --                  | --        |
| MA                | ++  | -     | +/-         | --  | -/+  | NA   | NA                  | NA        |
| Xp11.2 tRCC       | ++  | NA    | +/-         | --  | NA   | NA   | --                  | --        |
| t(6;11) RCC       | ++  | --    | --          | --  | --   | +    | --                  | --        |
| PRCC              | ++  | ++    | --          | --  | --   | --   | --                  | --        |

AMACR: Alpha-methylacyl-coenzyme A (CoA)-racemase; BRAF: V-Raf murine sarcoma viral oncogene homolog B1; CK7: Cytokeratin 7; IHC: Immunohistochemical; MA: Metanephric adenoma; NA: Not available; NR: Nephrogenic rest; PRCC: Papillary renal cell carcinoma (RCC); TFE3: Transcription factor binding to immunoglobulin heavy constant mu (IGHM) enhancer 3; TFEB: Transcription factor EB; tRCC: Translocation associated RCC; WT: Wilms tumor. *If positive, often only focally. Scoring: ++ >95% positive cases; + 76–95% positive cases; +/- 51–75% positive cases; - 5–25% positive cases; -- <5% positive cases.

Highly differentiated epithelial-type nephroblastoma can be formed of small, well-differentiated and closely packed tubules resembling MA, which consists of small, uniform, closely packed tubules and papillae, but the last one can be identified by the absence of capsule between the tumor and renal parenchyma, well defined delimitation from the renal parenchyma, the identification of small uniform cells with scant cytoplasm, bland nuclei, fine chromatin, the lack of mitotic activity, atypia and psammoma bodies [26, 31, 32]. MA also has an IHC pattern: CK7-negative, alpha-methylacyl-coenzyme A (CoA)-racemase (AMACR)-negative, WT1-positive, CD57-positive. The majority of MA carry a V-Raf murine sarcoma viral oncogene homolog B1 (BRAF) mutation (BRAF V600E most common), that can be demonstrated by immunohistochemistry. Nephroblastomas and MAs with overlapping HP and molecular characteristics were reported [23].

NB presents with high levels of catecholamines and on HP exam its cells present non-overlapping nuclei and coarse salt and pepper chromatin [28]. Both tumors can be positive for neuron-specific enolase (NSE) and cluster of differentiation (CD)56, but WT1 marker is negative in NB and NB84a is negative in nephroblastoma [26]. NB is also positive for synaptophysin, chromogranin A and paired-like homeobox 2b (PHOX2B). NB is PAX8-negative [30].

The epithelial component may contain the entire spectrum of differentiation from early stages of tubular formation with primitive epithelial rosette-like structures to differentiating tubules or glomeruli-like elements, showing different moments of nephrogenesis [23, 26, 28].

Pure epithelial type of nephroblastoma may be difficult to distinguish from hyperplastic periblolar NR, metanephrine adenoma (MA) and RCC [26]. Immunohistochemistry is useful in establishing a final diagnosis in most cases (Table 7) [29, 30].
present in some cases, mostly in tumors that have undergone preoperative chemotherapy [29, 33].

In the differential diagnosis of pure stratum type nephroblastoma, the following should be considered: CCSK, congenital mesoblastic nephroma and metanephric stromal tumor (MST) [26, 28]. Even though some renal masses with a mesenchymal pattern miss a specific IHC profile, a group of markers may be useful to distinguish the tumors (Table 8) [29]. In the stromal component of nephroblastoma, WT1 is often weak or absent [29].

### Table 8 – IHC characteristics of the most common renal tumors with a stromal pattern

| Stratal pattern | Immunohistochemistry |
|-----------------|----------------------|
|                 | WT1 | NGFR | BCOR | Cyclin D1 | INI1 | CD34 | BCL-2 |
|-----------------|-----|------|------|-----------|------|------|-------|
| WT              | +/- | -    | -    | +/-       | +    | +/-  | +     |
| CCSK            | ++  | ++   | +    | ++        | ++   | ++   | +/-   |
| Cellular/Mixed CMN | -   | -    | -    | ++        | ++   | --   | --    |
| Classic CMN     | --  | --   | -    | ++        | ++   | --   | --    |

CCSK is classically formed of bland ovoid cells with monomorphic nuclei with finely dispersed chromatin and small nucleoli organized in cords and nests disconnected by a chicken-wire vasculature, but there can be different patterns leading to an increased number of misdiagnoses [10, 29]. CCSK shows a distinctive IHC profile: cyclin D1, B-cell lymphoma 6 (BCL-6) corepressor (BCOR), nerve growth factor receptor (NGFR)-positive and WT1-negative [30].

Nephroblastomas with diffuse anaplasia or blastemal predominance are consecrated as high-risk tumors and need aggressive treatment [24]. We identified one case of focal anaplasia in a 3-year-old girl who presented with pulmonary metastases at diagnosis. Anaplastic nephroblastomas are defined by the presence of large, atypical, polyplar mitotic figures and significantly enlarged and hyperchromatic nuclei. Anaplasia may be focal or diffuse. Anaplasia is culpable for adverse response, mostly in advanced tumor stages [32, 33].

Preoperative chemotherapy may highly disturb the primary histopathology by reducing or enhancing some elements or by activating maturation [34, 35].

Clinical studies show that the results of patients with nephroblastomas are reliant to HP description [29]. Favorable histopathology is defined by the presence of all three HP components and the lack of diffuse anaplasia [16]. There are three HP risk groups: low risk: (completely necrotic nephroblastoma or cystic partially differentiated nephroblastoma), intermediate risk (regressive, epithelial, stromal, mixed, or focal anaplastic nephroblastoma) and high risk (blastemal or diffuse anaplastic nephroblastoma) [4, 29].

We identified three patients with favorable histopathology or low risk, 13 patients with intermediate risk and six patients with unfavorable histopathology or high risk. All three HP components of nephroblastomas present individual proliferation potential and respond differently to therapy [10].

### Radiological and HP staging and correlation

Staging criteria for nephroblastoma are based entirely on the anatomic extension of the mass, without taking into consideration genetic, biological, or molecular markers [14]. Stage is one of the most relevant therapeutic and prognostic criteria for kidney masses [4]. Staging is an important issue in multiple centers because kidney tumors are often large and usually it is hard to establish their relationship with normal anatomical elements [16].

Imagistic staging of nephroblastomas encompasses several steps. The radiologist needs to establish if the mass is limited to the kidney and if it can be totally removed with the surface of the renal capsule intact. In this case, the patient is regarded as stage I [36, 37]. Development of the mass outside the renal capsule obviates the classification of stage I [4]. Local extension into the neighboring tissues, renal vessels or inferior vena cava outside the kidney can be identified and, when grossly removed, the child is classified as stage II [12, 20]. The regional lymph nodes must be defined because this automatically places the patient into stage III. Lymph node status may be difficult to appreciate in large tumors obscuring lymph nodes or in case of lymph nodes larger than 1 cm that appeared secondary to the tumor, rather than infiltration [38]. Also, lymph nodes smaller than 1 cm can be infiltrated and not identified as atypical by radiological methods [15]. In the NWTS/COG protocols, staging includes intraoperative lymph node sampling because intraoperative macroscopic evaluation of positive ganglia is not reliable, false negative rates being as high as 31.3% [38]. Accurate interpretation of the emerging imaging data (tumor limited to the kidney, tumor extended beyond the kidney and lymph node involvement) permits the patient to be correctly classified into stages I–III [39].

Patients who present with systemic metastases usually in the lung, liver, bone or brain are classified as stage IV [14, 40]. Of these sites, the lung remains the most common, followed by the liver. It is critical to differentiate between lung nodules and atelectasis at the base of the lungs. Children with bilateral renal tumors at the diagnosis are classified as stage V. This is found in 5–7% of patients [2, 10]. The high-risk groups for bilateral nephroblastoma are those children who have multifocal lesions in their index kidney, children with nephroblastomatosis, those with associated congenital anomalies and children with a family history of nephroblastoma [4, 10].

At the moment, there are two important HP staging systems that are applied: an upfront surgery-based system developed by NWTS, and an upfront chemotherapy-based system developed by SIOP [16, 39]. Both staging systems have proven valuable in predicting outcomes [10]. Even if the architecture of nephroblastomas varies significantly, the tumor is allocated a HP type if more than two-thirds of the tumor tissue is of one element [4,
From a review of NWTSG studies, favorable and unfavorable HP subtypes are defined as the presence or the lack of anaplasia [16, 41]. The existence of anaplasia has been attributed to low tumor responsiveness to adjuvant chemotherapy [16]. The definition of focal anaplasia is based on topographical principles, requiring that anaplasia be limited to just a few specific areas [10, 35]. Also, the identification of HP subtypes is important, as risk stratification is also established by it [32, 42].

In our study, imagistic staging corresponded with HP staging in 82% of the patients. This number is comparable to the literature [38] and has been increasing with the improvement of imaging techniques [2], however CT scans are not yet sufficiently free of error to be able to correctly predict HP staging [43].

Other studies show various degrees of correlation between CT staging and HP staging of nephroblastoma, some being as low as 38% correspondence between the two [38]. In our study, the CT examination under staged two of nine (22%) local spread tumors (stage III), over staged one of two (50%) localized renal disease (stage I) tumors and over stages two of five (20%) local extension (stage II) tumors. Under staging tumors might be more significant, especially for stage III tumors, since complete excision is not achieved, therefore the patient has a significantly different prognosis [44].

We performed a Mann–Whitney U-test to evaluate the probability that staging discrepancies could be attributed to chance. The z-value was calculated to be 2.31, which is well over the 1.96 mark for a 5% chance. The probability that staging discrepancies could be attributed to low tumor responsiveness to adjuvant chemotherapy [16]. The definition of focal anaplasia is based on topographical principles, requiring that anaplasia be limited to just a few specific areas [10, 35]. Also, the identification of HP subtypes is important, as risk stratification is also established by it [32, 42].

In our study, imagistic staging corresponded with HP staging in 82% of the patients. This number is comparable to the literature [38] and has been increasing with the improvement of imaging techniques [2], however CT scans are not yet sufficiently free of error to be able to correctly predict HP staging [43].

Other studies show various degrees of correlation between CT staging and HP staging of nephroblastoma, some being as low as 38% correspondence between the two [38]. In our study, the CT examination under staged two of nine (22%) local spread tumors (stage III), over staged one of two (50%) localized renal disease (stage I) tumors and over staged one of five (20%) local extension (stage II) tumors. Under staging tumors might be more significant, especially for stage III tumors, since complete excision is not achieved, therefore the patient has a significantly different prognosis [44].

We performed a Mann–Whitney U-test to evaluate the probability that staging discrepancies could be attributed to chance. The z-value was calculated to be 2.31, which is well over the 1.96 mark for a 5% chance. This means that we can reasonably state that radiological evaluation of nephroblastoma cannot yet reliably predict HP staging.

Conclusions

Our study reviewed the medical records of 22 patients diagnosed with nephroblastoma between 2014 and 2020. This group provided a series of descriptive statistics of the demographics, imagistic and HP characteristics for the analysis. We found that the HP staging was over evaluated in 22% of cases and under evaluated at the same rate by the imagistic staging system. We compared the imagistic and HP staging distributions via a Mann–Whitney U-test and concluded that imagistic staging cannot be yet used to predict HP staging with satisfactory precision. Differential diagnosis is challenging especially in cases of monophasic pattern nephroblastomas, immunohistochemistry having a pivotal role for correct diagnosis and subsequent therapeutic management.

Conflict of interests

The authors declare that they have no conflict of interests.

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