

HISTOLOGICAL RESPONSE OF WILMS TUMOUR TO PREOPERATIVE CHEMOTHERAPY IN CHILDREN IN RWANDA

Sylvie Umuganwa, MD

Master of Medicine (Anatomical Pathology) Dissertation
University of Rwanda

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By

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A Dissertation submitted in partial fulfilment of the requirements for the Degree of Master of Medicine (Anatomical Pathology) of the University of Rwanda.

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December 2021

CERTIFICATION FOR AWARD

The undersigned certify that they have read and hereby recommend for examination by the University of Rwanda a dissertation entitled ‘’**Histological response of Wilms tumour to preoperative chemotherapy in children in Rwanda’’** in partial fulfilment of the requirements for the Degree of Master of Medicine (Anatomical Pathology) of the University of Rwanda.



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DECLARATION AND COPYRIGHT

I, Sylvie Umuganwa, declare that this dissertation is my own original work except where specifically acknowledged and it has not been presented to any other University for similar or any other degree award.

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Thank you to my colleagues, the Anatomical Pathology residents.

Thank you to my family.

Last but not least, I thank the Almighty God.

DEDICATION

To Michèle Kayiganwa

ABSTRACT

Renal tumours constitute about 8% of all tumours in the first 15 years of life. In this age range, Wilms tumour is the most common occupying 85% of all cases. Currently in Rwanda, Wilms tumour is the most common paediatric renal tumour. It comprises 6% of all paediatric malignancies and 90% are diagnosed before the age of three. The survival rates of Wilms tumour are still low in most African countries Rwanda included. Its histological responsiveness is known to affect prognosis. The aim of our study was to obtain accurate data regarding this histological response of Wilms tumour after administration of preoperative chemotherapy in children in our country by determining the frequency of the viable tumour, its subtypes and if sex and age influence the tumour's histological response to the preoperative chemotherapy.

After acquiring the ethical approval from the Institutional review board (IRB) of the University of Rwanda (UR) College of Medicine and health sciences (CMHS), permissions from the institutions involved in the study were also obtained before data was collected. A total of 96 cases were found over a period of 7 years, from 2013 to 2020. Their haematoxylin and eosin (H&E)-stained glass slides were retrieved and reviewed by two independent pathologists for confirmation of the diagnosis including the presence of the viable tumour, its type, its subtypes as well as the histological response. We also looked at the degree of necrosis, risk group and stage. The recorded data was analysed using statistical product and service solutions (SPSS) 26.0, $p<0.05$ was considered as showing a statistically significant association.

Of the 96 cases collected, 51 (53.1%) were females while 45 (46.9%) were males. The 2–5 age group was the most represented (65.6%) with a median of 3 years. Viable tumour was seen in 81 cases (84.4%) and almost all of them were solid (96.3%). The mixed subtype was the most predominant with 45.7% while the epithelial subtype was seen the least at 7.4%. Necrosis was present in almost all cases (99%) and the estimated degree of necrosis of 10-30% was the most seen (44.2% of cases). The intermediate risk group predominated at 62.5% as well as stage I at 64.5%. We found that males were more likely to have a higher stage (III and above) compared to females (p-value=0.022, OR=4.85, 95% CI= 1.25-18.76).

Our study showed that, in our settings, the degree of necrosis in Wilms tumours after administration of preoperative chemotherapy is insufficient. This merits further investigations for validation and eventually review of the existing treatment protocols.

Key words: Wilms' tumour, pre-operative chemotherapy, histological response.

LIST OF ABBREVIATIONS

CAP: College of American Pathologists

CHUB: University Teaching Hospital of Butare (Centre Hospitalier Universitaire de Butare)

CHUK: University Teaching Hospital of Kigali (Centre Hospitalier Universitaire de Kigali)

CMHS: College of Medicine and Health Sciences

COG: Children's Oncology Group

FFPE: Formalin-fixed paraffin-embedded

H&E: Haematoxylin & Eosin

HRH: Human resource for health

IRB: Institutional Review Board

KFH: King Faisal Hospital

LMIC: Low- and middle-income countries

MOH: Ministry of Health

NWTSG: National Wilms tumour study group

RMH: Rwanda Military Hospital

SIOP: International society of paediatric oncology

UR: University of Rwanda

USA: United States of America

WHO: World Health Organization

WT: Wilms tumour

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- Data collection form
- Ethical approval from the CMHS Institutional Review Board
- Permission to collect data at CHUK
- Permission to collect data at CHUB
- Permission to collect data at KFH

CHAPTER I. INTRODUCTION

1.1 Background

It was estimated that there were about 12.7 million new cancer cases and 7.6 million cancer deaths in 2008 worldwide (1). In 2020, the estimate was between 15 and 17 million new cases of cancer, 60% of which were in developing countries (2). The magnitude of the global burden of childhood and adolescent cancer remains poorly quantified (3). There are no global estimates of incidence, survival, and mortality for children with cancer in most low- and middle-income countries mostly because of the paucity of high-quality cancer registration and vital statistics data (3). Overall, children with cancer represent a small proportion, approximately 1% of all cancers diagnosed worldwide each year (3). Studies have shown that paediatric cancer incidence rates have been steadily increasing (4). In developed countries, cancer is the second commonest cause of death in children (5). Furthermore, across the world, cancer is the leading cause of mortality in children and adolescents, it is also the leading medical cause of death in both males and females from the age of 1 to 34 (6).

Most of the current literature evaluates paediatric cancer incidence in the 0–14 or 0–19 years age range (4). Although currently known risk factors account for a small percentage of the overall disease burden, it is plausible that known risk factors such as birth weight, parental age, congenital abnormalities, chronic infections, and genetic syndromes may differ in infants and young children compared to adolescents (4).

A study which collected data from 18 sub-Saharan African countries found that 1.4 % cancer cases in Ghana and 10% in Rwanda were childhood cancers (7). In West African countries such as Ivory Coast, Mali and Ghana, Non-Hodgkin lymphoma was found to be the most common with 73.6%, 32.7% and 53.6% respectively (7). Among children in Mozambique, Kaposi sarcoma was the most predominant (15.8%) malignancy (7). This was also the case in Malawi (12.4%), Zambia (15.6%) and Uganda (22.0%) (7). Two centres in Kenya registered mainly Burkitt lymphoma (25.1 and 37.1%) (7). Retinoblastoma was recorded with the highest proportion in Congo at 20.1% (7). The study found Nephroblastoma to be the most prevalent solid tumour in many African countries with over 10% of all paediatric cancers (Congo 15.5%, Mali 17.6%, Rwanda 21.3%, Ivory Coast 14.5%, Senegal 22%) (7). We, however, have to bear in mind that most of the mentioned countries do not have well-established cancer registries, these frequencies are estimates or small reports from single/few institutions.

Paediatric renal tumours compose one of the major groups of childhood solid neoplasms (8). In European populations, 97% of the malignant renal neoplasms are Wilms tumours (WT) (8). The majority, approximately 80% occur before the age of 5 years implying a genetic element in the aetiology (8). The highest incidence of Wilms tumour occurs in some black populations in Africa and the USA, with rates commonly in the range of 9–12 per million (9). Among white populations, the incidence was found to be generally somehow lower, typically 6–10 per million (9). Lower rates, under 5 per million are found throughout East Asia (10). Generally, the incidence of malignant paediatric tumours is difficult to estimate in Africa, due to various factors, the lack of vital hospital statistics and cancer registries in most countries being the main reason (11). In Rwanda, Wilms tumour is the commonest childhood cancer (12). Looking at the survival rates of Wilms tumour, 5-year survival of early stage WT is above 90% in high income countries while survival rates in African settings are generally less than 45% (10) (13).

The management of Wilms tumour involves surgery, pre-operative and post-operative chemotherapy as well as radiotherapy (14). Worldwide, two main strategies are used; the International Society of Paediatric Oncology (SIOP) which recommends chemotherapy before surgery and the children's oncology group (COG) that relies on primary nephrectomy followed by post-operative chemotherapy (14). In Rwanda, SIOP protocol is used and depending on the results of the histopathology, postoperative chemotherapy is then given accordingly. According to the SIOP, pre-operative chemotherapy for unilateral localised Wilms tumour is 4 weeks of vincristine and dactinomycin (15). For those with bilateral tumours, vincristine-dactinomycin are given for 9-12 weeks with the addition of doxorubicin in some patients (15). Patients with metastasis receive vincristine-dactinomycin for 6 weeks with doxorubicin on weeks 1 and 5 (15). Elsewhere mostly in the developed countries, after administration of preoperative chemotherapy, the distribution of the viable tumour subtypes as well as factors influencing or associated with the tumour's histological response have been documented (16). The aim of our study is to evaluate these parameters in the patients in our settings.

1.2 Problem statement

Cancer is a major cause of death in children worldwide (6). This is even more so in low-income countries where cancer care and life-saving therapies are still not fully accessible (6).
In Rwanda, as in many other sub-Saharan countries, cancer care has improved but is still a relatively new and limited discipline (3). Of all the paediatric malignancies diagnosed in our settings, Wilms tumour is the commonest childhood renal tumour affecting mainly children

from 1- to 5-year old with a peak between 2 to 3 years (12)(17). Wilms tumour can occur in adolescents and adults although this is rare representing below 1% of all renal tumours (18). Today, Rwanda has made progress in reducing infection-related childhood deaths over the last two decades (12). More efforts are now directed towards addressing the burden of non-communicable diseases including cancers (12). In addition to preoperative chemotherapy for the treatment of Wilms tumour in Rwanda, radiotherapy has been recently introduced and it is also administered following the SIOP guidelines.

When a child is suspected to have nephroblastoma, he/she receives neoadjuvant chemotherapy followed by surgery then the specimen is sent for histopathology analysis. Preoperative treatment with chemotherapy was introduced in 2012 and up till now it is given at one cancer centre, the Butaro Cancer centre of excellence. The regimen given consists of vincristine and actinomycin D for four weeks for localised stage I to III disease and six weeks of vincristine, actinomycin D with the addition of doxorubicin for metastatic stage IV and V disease (10). It is of prognostic importance to know the tumour histological responsiveness to preoperative chemotherapy (8). In case a tumour remains viable after chemotherapy and where the blastemal component is predominant, it is linked with unfavourable outcome, therefore, after neoadjuvant chemotherapy, the tumour response may guide subsequent therapeutic strategy (19). Gordon M. Vujanic *et al* compared WT's where instant surgery was performed versus those that received preoperative chemotherapy (16). They found that the blastemal component was very responsive to preoperative chemotherapy hence being the least common type (16). They also found that preoperative chemotherapy helps in identification of prognostically important WT types and it downstages tumours (16). On the other hand, preoperative chemotherapy was found to make subtyping and staging more difficult mostly due to chemotherapy-induced changes (16).

The survival of children with Wilms tumour in most developed countries is much higher compared with low- and middle-income countries (LMICs) specifically in Africa where it has been estimated to be less than 45% (10) and less than 50% particularly in low-income countries in sub-Saharan Africa (20). Some African countries have reported survival rates of 11% (Sudan), 46% (Malawi), 41% (Kenya) and 43% (Cameroon) (20). These rates were all performed over a two-year period (20). In Rwanda, in a study in which 53 patients with Wilms tumour were followed, 30.2% died within 2 years (10).

Although traditionally the response to chemotherapy is assessed by imaging by comparing tumour size before and after administration of chemotherapy (21), the histological responsiveness is what is known to specifically affect prognosis (16). When this is considered and added to the low survival rates seen in our settings, the aim of our study was to assess this histological response in our patients.

1.3 Research question

What is the histological response of Wilms tumour to neoadjuvant chemotherapy in children in Rwanda?

1.4 Objectives

1.4.1 Main objective

To describe the histological response of Wilms tumour to neoadjuvant chemotherapy in children in Rwanda.

1.4.2 Specific objective

- To determine the frequency of the viable Wilms' tumour after administration of preoperative chemotherapy.
- To determine the subtypes of the viable Wilms' tumour after administration of preoperative chemotherapy.
- To identify if age or sex influence Wilms tumour histological response to preoperative chemotherapy.

CHAPTER II. LITERATURE REVIEW

A number of different entities of renal tumours in childhood range from benign to malignant including Wilms tumour, cystic nephroma, renal cell carcinoma of childhood, metanephric tumours, clear cell carcinoma of the kidney, rhabdoid tumour of the kidney, mesoblastic nephroma, primary intrarenal neuroblastoma, primitive neuroectodermal tumour of the kidney, desmoplastic small round cell tumour of the kidney, synovial sarcoma of the kidney and anaplastic sarcoma of the kidney (8).

The kidneys are retroperitoneal and located from T12 to L3 vertebrae when in the upright position(22). The right kidney is slightly lower than the left due to the size of the right lobe of the liver (22). The kidney is invested by a firm fibrous capsule that is surrounded by the renal fascia (22). On its medial border is the hilum through which the ureter and renal vessels plus nerves enter (22). It consists of the medulla and the cortex which contains the nephrons and these are the functional units of the kidney (22). The kidney's functions include excreting waste products, regulating body fluid and solute balance, regulating blood pressure, and secreting hormones (23).

During embryologic stage, the foetal kidney originates from the ureteric bud and the metanephric mesenchyme/blastema (18). By 36-weeks' gestation, the blastemal has usually disappeared, but at birth approximately 1% of infants retain residual blastema within their kidney (18). These cells are described as nephrogenic rests and a nephrogenic rest is defined as 'nests of abnormally persistent nephrogenic cells which may be induced to form a Wilms tumour (18).

Wilms tumour is a malignant renal neoplasm (8). It affects one in 10,000 children and above 80% of them are diagnosed before the age of 5 years (8). The median age at diagnosis is 3.5 years (8). It shows a slight female preponderance in Caucasian children but a higher incidence in boys compared to girls has been observed among East-Asian children (18). The highest rates are recorded in children of black descent (18). Wilms tumour can sometimes be bilateral (18).

Most cases (90%) are sporadic while the remainder may be associated with genetic syndromes (18). Over 50 such syndromes have been described but those commonly seen are WAGR (Wilms' tumour, aniridia, genitourinary abnormalities, and mental retardation), Denys-Drash and Beckwith-Wiedemann syndromes (18).

WT is considered a genetically heterogeneous disease that arises in the setting of specific and consistent “hot spot” alterations in predisposing genetic loci, which theoretically could vary with race and, therefore, drive the observed disparate incidences (24). Specifically, mutations in WT1, CTNNB1, WTX, and IGF2 have been shown to occur commonly in WT (24). Some markers have been shown to be associated with adverse clinical disease features, for example accumulation of the tumour suppressor protein p53 is associated with treatment resistance while a proliferative index greater than 5% has been associated with greatly increased disease progression and decreased tumour-specific survival (25).

Patients show non-specific features including an abdominal mass, abdominal pain, haematuria and hypertension (26). Atypical presentation can occur in less than 10% of cases due to compression of surrounding organs or tumour extension (26). During evaluation of Wilms tumour, an ultrasound scan is a useful primordial investigation, it confirms the existence of an intrarenal mass, its extension and whether there is involvement of the contralateral kidney or not (26). A computed tomography (CT) scan is now considered standard practice or Magnetic Resonance Imaging (MRI) in children with a suspected abdominal mass (26). The decision regarding CT versus MRI is at the discretion of the treating institution, especially for locoregional staging, because they have been shown to have equivalent diagnostic performance (26). The chest should also be assessed because it is the most predominant site of metastatic spread (18).

Preoperative chemotherapy has proved to play an important role in the treatment of most paediatric solid tumours including Wilms. It facilitates complete surgical removal of a shrunken tumour, mutilation caused by surgical procedures is minimized or avoided and micro metastases not visible at diagnosis are treated as early as possible (27). In addition, the response to therapy can be evaluated by the percentage of tumour induced necrosis in the histologic specimen after surgery (27).

Post therapy tumour response is classified into low risk, intermediate risk and high risk by the SIOP (19). In low risk, there is no viable tumour present, only scattered nephroblastic tubules which may represent residual nephrogenic rests (19). In intermediate risk, viable tumour is present and it can comprise <33% of the mass regardless of histology or it can comprise >33% of the mass with blastemal histology present in <66% of the viable tumour (19). In high risk, the viable tumour is >33% and the blastemal histology is >66% of the viable tumour (19).

Response to chemotherapy cannot be predicted with certainty and the histopathologic changes vary from one person to another although patient's age, tumour stage and tumour biological factors have been shown to play a role (28)(29).

Chemotherapy changes seen histologically include necrosis, fibrosis, foamy and hemosiderin-laden macrophages (8). In the course of chemotherapy, the rapidly dividing cells mainly the primitive epithelial or blastemal components respond while the mature stromal and epithelial components may not, hence, these stromal components may seem more prominent in the post chemotherapy specimen (8).

In a study done in the USA, the post chemotherapy Wilms tumours exhibited variable histology fundamentally characterized by the formation of abortive or embryonic glomerular or tubular structures surrounded by immature stroma (30). Blastemal elements were also present and they showed transition into epithelial and stromal elements (30). This admixture of epithelial, stromal, and blastematous elements, typical of Wilms tumours, was present in all the cases they studied except one which was considered unaffected by preoperative chemotherapy because it showed no changes (30). Other histologic changes included rhabdomyoblastic component which also showed no evidence of chemotherapeutic effect, numerous histiocytes and necrosis (30). Stromal changes seen included oedema, fibrovascular to fibroblastic tissue with numerous capillaries (30). Vascular changes observed were fibrinoid necrosis, hyalinization, haemorrhage and acute inflammatory infiltrates in the vessel wall as well as thrombosis (30).

These findings are similar to those found in a study done in Indian children with Wilms tumours who received preoperative chemotherapy where the mesenchymal component also showed rhabdoid differentiation in some cases and chondroid in others (28). The epithelial component however, showed squamous differentiation while the blastemal component decreased markedly and disappearing completely in some patients (28).

In more recent studies done in Europe, the proportion of necrosis seen in the post chemotherapy specimen was 80% (31). The specimens with least necrosis were of mixed, blastemal, stromal and epithelial types (31).

CHAPTER III. RESEARCH METHODS

3.1 Type of study

This was a retrospective descriptive study. It covered a period of 7 years, from October 2013 to October 2020.

3.2 Study site

The study was done in the Anatomical Pathology departments of the University Teaching Hospital of Kigali (CHUK), University Teaching Hospital of Butare (CHUB) and King Faisal Hospital, Kigali (KFH).

3.3 Study population

All patients aged between 0 and 15 years diagnosed with Wilms tumour and who received preoperative chemotherapy during the period of October 2013 to October 2020.

3.4 Selection of study population

3.4.1 Inclusion criteria

- All patients aged between 0 and 15 diagnosed with Wilms tumour and who received preoperative chemotherapy.
- Resection specimens whose FFPE tissue blocks, H&E-stained glass slides (or either) as well as clinical information could be found.

3.4.2 Exclusion criteria

- Patients diagnosed with Wilms tumour but who are not in the indicated age range.
- Patients diagnosed with Wilms tumour but did not receive preoperative chemotherapy.

3.5 Sample size

We calculated the sample size using the following formula:

$S = \frac{Z^2 \times P \times (1-P)}{E^2}$, where:

S: Sample size

Z: Z score (1.96 for 95% confidence interval)

P: Population proportion

E: Margin of error (5%)

We conducted a small pilot study at CHUK for the year 2013; we looked at all the biopsies received of children aged 0-15 years. The rate of children with Wilms tumour and who had preoperative chemotherapy administered was 6%. Using this rate, we calculated the sample size as follows:

$$S = \frac{(1.96)^2 \times 0.06 \times (1-0.06)}{(0.05)^2} = 86$$

3.6 Study procedures

3.6.1 Data collection

We used the histopathology archives at the study sites to retrieve all the cases of children with Wilms tumour in the age range indicated and who received preoperative chemotherapy within the mentioned study period. Every case's clinical information, demographic data and Formalin fixed paraffin embedded (FFPE) tissue blocks as well as previous pathology reports were retrieved. Haematoxylin and eosin (H&E) stained glass slides were reviewed. The diagnosis rendered including percentage of the viable tumour, its subtype and overall histological response was confirmed by two blinded pathologists. During microscopic review when assessing the pre-treated Wilms tumours, in case only necrosis/regressive changes were present with no viable tumour identified it was considered as completely necrotic WT (32). In case regressive changes comprised above two-thirds of the tumour mass, it was a regressive type (32). In case regressive changes comprised below two-thirds of the tumour mass, the viable tumour was sub-classified based on the viable components present (stromal, blastemal, epithelial or mixed type) (32). In case of focal anaplasia, the tumour is still sub-classified based on other components (32). In case of disagreements, a consensus was reached. For each case, we recorded:

- Demographic information (age, sex, province of origin)
- Clinical information
- Histology results (chemotherapy response, risk group, stage)

A detailed data collection form was used to collect the information.

In case the original H&E-stained slides for a particular case were of poor quality or could not be found, the FFPE tissue blocks were used to re-cut and re-stain new slides.

3.6.2 Data analysis

Information collected using the data collection sheet was entered into excel from where it was analysed using SPSS (IBM Corporation, New York, 10504-1722) version 26. The demographic and histopathological data were analysed statistically in terms of frequency and means. The results are presented in tables.

Cross tabulations then logistic regression analysis with odds ratio (OR) and their 95% confidence interval (CI) were used for analysing relationships. Statistical significance was assessed based on a p-value < 0.05 and the 95% CI of the OR.

3.7 Ethical consideration

3.7.1 Confidentiality

Each case was given a code number corresponding to but different from the histopathology case number. This information was stored on a personal password-protected computer.

3.7.2 Ethical approval

The department of Clinical Biology of the College of Medicine and Health Sciences (CMHS) at the University of Rwanda reviewed the scientific aspect of the study and granted their approval (approval notice n^{o} 075/CMHS IRB/2020).

We also requested and were granted permission from the above-mentioned hospitals where data were collected.

3.8 Strengths and limitations of the study

3.8.1 Strengths

This study highlighted the response of Wilms tumour to preoperative chemotherapy in the patients in our settings, information that was not known before and which can be used by future researchers.

3.8.2 Problems and limitations

Preoperative chemotherapy highly modifies the histological characteristics of Wilms tumour. This is a universal challenge observed elsewhere (32).

CHAPTER IV. RESULTS

4.1 Patients' demographics

We obtained a total number of 96 cases of patients who had Wilms tumour and who had received preoperative chemotherapy. 90 were from CHUK, 5 from KFH and one case was from CHUB. We were able to retrieve the slides of all the cases and those that were not found, new ones were recut and re-stained from their respective FFPE tissue blocks.

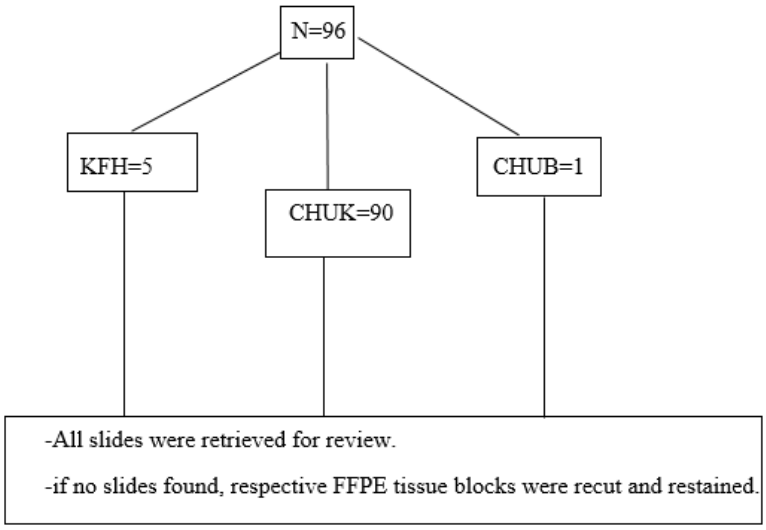


Figure 1. Algorithm summarizing how participants were recruited

CHUB: University Teaching Hospital of Butare, CHUK: University Teaching Hospital of Kigali, KFH: King Faisal Hospital- Kigali

The patients' files were also consulted whenever more clarifications were needed; this was mostly seen for staging whereby important information required (tumour spillage during surgery, involvement of the vena cava) was sometimes not mentioned on the pathology request form or even seen in the electronic record system.

Table 1 shows that females (53.1%) were more than males (46.9%). Most patients (65.6%) were between 2 and 5 years with 3 as the median age. The Western province was the main region of residence with 28.1%. Kigali city, the northern and eastern provinces had a similar distribution of 15.6%.

Table 1. Distribution of demographic features of study participants

Characteristics	n	%
Sex (n=96)		
Female	51	53.1
Male	45	46.9
Age (median=3; mean= 3.86, range= 9 months-13 years) years (n=96)		
≤1 year	12	12.5
2-5 years	63	65.6
6-11years	20	20.8
12 years and above	1	1.0
Residence (n=96)		
Western Province	27	28.1
Southern Province	18	18.8
Kigali City	15	15.6
Northern Province	15	15.6
Eastern Province	15	15.6
Other (Foreigners)	6	6.3

4.2 Tumour viability

In table 2, we describe the viable tumour based on whether it was cystic or solid as well as the different subtypes. Of the 96 cases, 84.4% showed a viable tumour. Among the histological subtypes, the mixed type (regressive changes comprised below two-thirds of the tumour mass and the stromal, epithelial and blastemal components each ranging from 0-65%) was the most predominant. The least predominant was the epithelial subtype.

Figure 1 is a photomicrograph of the different subtypes on H&E-stained sections.

Table 2. Tumour viability

Characteristics	n	%
Viable tumour (n=96)		
Yes	81	84.4
No	15	15.6
Gross appearance of viable tumour (n=81)		
Cystic	3	3.7
Solid	78	96.3
Histological subtype of viable tumour (n=81)		
Mixed type	37	45.7
Blastemal predominant	16	19.8
Regressive	15	18.5
Mesenchymal predominant	7	8.6
Epithelial predominant	6	7.4

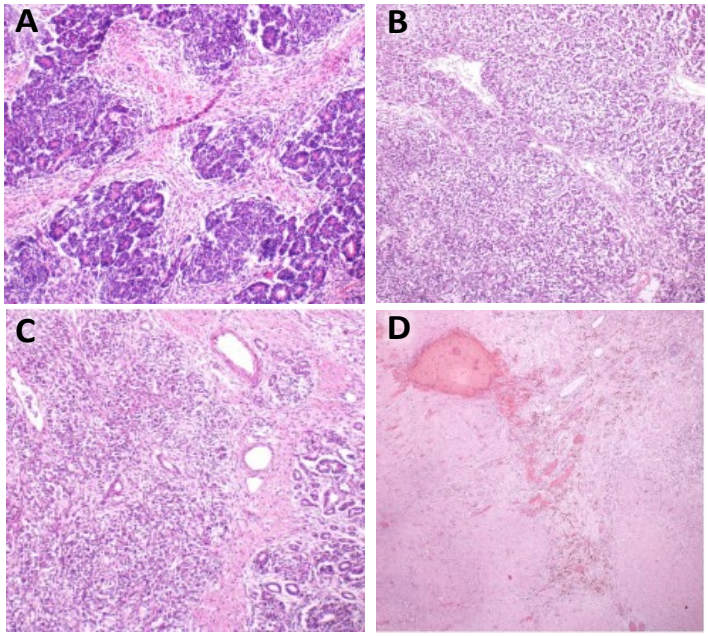


Figure 2. Different subtypes of viable tumour on haematoxylin and eosin (H&E)-stains

Photomicrograph A: Blastemal predominant subtype with rosette formation (H&E stain, 100X), B: Epithelial predominant subtype (H&E stain, 100X), C: Mixed subtype (H&E stain, 100X), D: Regressive subtype (H&E stain, 100X)

4.3 Histological response

Table 3 describes the degree of necrosis, the risk group and the stage. Necrosis was mostly seen between 10 and 30% (44.2%). Most of the cases were of intermediate risk (62.5%) and in stage I (64.5%).

Table 3. Histological response

Characteristics	n	%
Necrosis (n=96)		
Yes	95	99
No	1	1
Estimated percentage of necrosis (n=95)		
<10%	14	14.7
10-30%	42	44.2
31-60%	15	15.8
61-89%	10	10.5
≥ 90%	14	14.7
Risk group (n=96)		
Low risk	20	20.8
Intermediate risk	60	62.5
High risk	16	16.7
Stage (n=93)		
Stage I	60	64.5
Stage II	19	20.4
Stage III	11	11.8
Stage IV	1	1.1
Stage V	2	2.2

4.4 Association between sex, age and histological response

Table 4 shows the association between sex, age and histological response (risk group, estimated percentage of necrosis, viable tumour subtype and stage).

From the table, we see that male patients were more likely to have an intermediate/high risk group compared to the females but the difference was not statistically significant (p=0.489, OR=1.42, 95% CI=0.52-3.87). Regarding the age, children aged 6 years and above were 3 times tended to have an intermediate/high risk group compared to those below 6 years of age but this too was not statistically significant (p=0.165, OR=3, 95% CI= 0.64-11.1).

For the stage, male patients were more likely to have a higher stage (stage III and above) compared to female patients, and this difference was statistically significant (p=0.022, OR=4.85, 95% CI= 1.25-18.76). Those aged 6 and above were 2.18 times more likely to have a higher stage than those below 6 years of age but there was no statistical significance (p=0.21, OR=2.18, 95% CI= 0.64-7.43). Three cases could not be staged because lymph node metastasis was suspected, however, appropriate immunohistochemistry studies could not be done for confirmation.

Table 4. Association between sex, age and histological response

Risk factor	Group 1	Group 2	OR (95% CI)	p-value
Risk group	***Low***	***Intermediate/High***		
Sex				
Female	12	39	1.42 (0.52-3.87)	0.489
Male	8	37		
Age				
<6 years	18	57	3.00 (0.64-11.1)	0.165
≥6 years	2	20		
Percentage of necrosis	***≤ 30%***	***> 30%***		
Sex				
Female	27	24	1.61 (0.71-3.66)	0.255
Male	29	16		
Age				
<6 years	42	33	1.57 (0.57-4.34)	0.383
≥6 years	14	7		
Viable tumour subtype	***Blastemal/Mixed***	***Others***		
Sex				
Female	25	17	1.73 (0.68-4.39)	0.248
Male	28	11		
Age				
<6 years	39	23	1.65 (0.53-5.18)	0.39
≥6 years	14	5		
Stage	***Stage I-II***	***Stage III-V***		
Sex				
Female	45	3	4.85 (**1.25-18.76**)	**0.022**
Male	34	11		
Age				
<6 years	63	9	2.18 (0.64-7.43)	0.21
≥6 years	17	5		

OR: odds ratio, CI: confidence interval

CHAPTER V. DISCUSSION

In most developed countries, data describing Wilms tumour epidemiology and response to treatment is well established and has been updated over the years. In Rwanda, little information is known. Based on the low survival rates in most African settings, Rwanda included, our study intended to highlight what the histological response to preoperative chemotherapy entailed. With this new information, correlations can be made between the histological response and the prognosis for even more improved patient care. It is also of great importance to know this in our settings because it has an implication in the therapeutics as well as further planning in diagnosis and detection.

Our patients were from different parts of the country, the Western province at 28.1% was followed by the Southern Province at 18.8%. The eastern and northern provinces and Kigali city had a similar number of patients and each occupies 15.6%. The remaining percentage (6.3%) represents patients from outside Rwanda who mostly come from the neighbouring countries of Burundi and the Democratic Republic of Congo. At this point, no scientific explanation has been elaborated regarding this distribution. This calls for further investigations to identify and take on the factors that may be responsible for this presentation.

All the specimens in the study were radical nephrectomies done in patients who had received preoperative chemotherapy as we use the SIOP protocol. We found a small female predominance in our patients where they were 53.1% and males were 46.9%. This slight female preponderance has been described in general in populations in Eastern Asia (33), it was also reported in a study evaluating the survival and mortality rates in Southern and Eastern European countries where the females occupied 52% (34).

We found that Wilms tumour occurred predominantly in children aged ≤5 years (78.1%), the majority of patients being those aged 2 to 5 (65.6%) with a median age of 3 years. These findings reflect those reported by Szychot *et al.* in their review article on Wilms tumour done in the United Kingdom where they described the median age for the diagnosis of Wilms tumour to be 3.5 years and more than 80% of cases diagnosed in children below 5 years of age (18).

The frequency of the viable tumour after preoperative chemotherapy in our study was 84.4% (81 patients), whereas in 15.6% (15 patients), there was complete necrosis with no viable tumour present. In their study, Taskinen *et al.* found the percentage of fully necrotic tumours to be 10% after preoperative chemotherapy at Helsinki University hospital, Finland (31).

Regarding the subtypes of the viable tumour, we found the most predominant to be the mixed subtype with 45.7% followed by the blastemal predominant with 19.8% then regressive with 18.5%, mesenchymal with 8.6% and lastly epithelial with 7.4%. These findings are similar to those Kaste *et al.* reported whereby after administration of chemotherapy the mixed subtype was also found to be the most predominant and the epithelial the least predominant (35). The mixed subtype was again found to have a higher percentage compared to the other subtypes by Taskinen *et al.* (31). The percentage of the epithelial subtype was again reported to be the least predominant after administration of chemotherapy by Vujanic *et al.*, they however reported the regressive type to be most predominant (32). Preoperative chemotherapy is known to destruct blastema and epithelial elements most (32) which explains why epithelial predominant subtypes are found in small percentages after preoperative chemotherapy administration. This, however, seems to be different in the blastemal predominant components in our settings which were found at a moderately higher proportion compared to other studies warranting further research into the matter.

Necrosis was seen at 99% (95 patients) and 44.2% (42 patients) showed estimated necrosis between 10 and 30%. This may cast a light on why in our study we found the mixed and blastemal predominant subtypes to be more prevalent as observed in non-pre-treated Wilms tumours (32). In light of this distribution of necrosis in our patients, we speculate that the amount seen in the sections microscopically is small (10-30%) compared to elsewhere where it reached 80% (31). With this in mind and the relatively poor prognosis and survival of Wilms tumour in our settings (10), it may indicate a suboptimal response to preoperative chemotherapy. In childhood solid neoplasms, tumour necrosis following neoadjuvant chemotherapy is an important predictor of disease outcome because survival rates are better in good responders to neoadjuvant chemotherapy with high tumour necrosis than those with poor response (36).

Intermediate and low risk groups combined accounted for 80% where the former was 60% and the latter 20%. This identifies with what has been reported describing the intermediate risk group as the most common of the three risk groups (37). For the staging, more than half (84.9%) were stages I and II with 64.5% accounting for stage I and 20.4% for stage II. Preoperative chemotherapy is known to promote a favourable stage distribution (16), these findings mirror this fact. Similar findings were identified in the United Kingdom Wilms tumour study 3 (UKW3) experience where out of 102 patients who received preoperative chemotherapy, 83% of the patients were those of stages I and II (16).

In our study, males were found to be 4.885 times more likely to have a higher stage (stages III-V) compared to the females and this difference was statistically significant. The stage has been described as the sole predictor of outcome (13). On the other hand, the patient's sex does not affect the outcome (38). Further research to validate our finding is encouraged because if males are more likely to have a higher stage, then this would imply that there is a probability that they may do poorly compared to the females in our settings. The other comparisons of age and sex with risk group as well as age with stage were not statistically significant. This in a way echo what Aronson *et al.* reported whereby in their analysis of comparison of age and stage showed that age had no significant impact on prognosis (39).

CHAPTER VI. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

To the best of our knowledge, this is the first study assessing the histopathological response of Wilms tumour to preoperative chemotherapy in children in Rwanda. After the above findings, it is obvious that in our patients the response may be regarded as suboptimal compared to elsewhere. In our opinion, this finding is of great significance because it can be the basis of change and advancement in the management of Wilms tumour in our settings. This novel finding in Rwanda needs to be validated through further studies. In the end, a plan to review existing treatment regimens merits to be considered.

Furthermore, this study has also showed that in our settings some of the information known worldwide regarding Wilms tumour is as well true for our population. We observed that the predominantly affected age group, risk group and stage found after preoperative chemotherapy administration are similar to other geographic regions.

6.2 Recommendations

Based on the findings from our study, we recommend the following;

1. Further studies to establish evidence-based explanations as to why in our settings mixed and blastemal subtypes were most predominant after administration of preoperative chemotherapy as this is unusual compared to other studies.
2. We realise that there is room for genotyping in our patients, this would provide insight into why we see a small amount of necrosis despite preoperative chemotherapy administration. The possibility that our patients may carry markers associated with treatment resistance or even other markers associated with adverse outcome cannot be passed on.
3. The largest number of patients was from the Western province; this can also be further investigated to establish if there is any relationship with the disease.
4. We found that males are more likely to have a higher stage compared to females, a finding that needs to be validated.
5. Communication between surgeons and pathologists should be reinforced and it should be done in a consistent and efficient manner to solve the problem of missing important information on the pathologists' side.

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APPENDICES

Data collection form

Form number: ☐ Research code: ☐

Demographic data

1. Age: ☐ 2. Sex: ☐

Residence**:**

Kigali City ☐ East ☐ West ☐ North ☐ South ☐ Other ☐

Clinical data

1. Hospital patient was enrolled in**:**

CHUK ☐ CHUB ☐ KFH ☐ RMH ☐

2. Year of diagnosis/surgery:

Histopathology result

1. Chemotherapy response:

I. Necrosis

Yes ☐ No ☐

II. If yes;

☐ <10%

☐ 10-30%

☐ 31-60%

☐ 61-89%

☐ ≥ 90%

III. Viable tumour

Yes ☐ No ☐

III. If yes;

Cystic ☐ Solid ☐

IV. Subtype of viable tumour;

☐ Epithelial predominant

☐ Blastemal predominant

☐ Mesenchymal predominant

☐ Mixed type

2. Risk group:

☐ Low risk ☐ Intermediate risk ☐ High risk

3. Stage of tumour (post chemotherapy):

☐ Stage I

☐ Stage II

☐ Stage III

☐ Stage IV

☐ Stage V

CMHS INSTITUTIONAL REVIEW BOARD (IRB)

Kigali, 20th May 2020

Dr. Sylvie UMUGANWA
School of Medicine and Pharmacy, CMHS, UR

Approval Notice: No 075/CMHS IRB/2020

Your Project Title ***"Histological Response of Wilms' Tumor to Preoperative Chemotherapy in Children Aged 0-15 Years in Rwanda"*** has been evaluated by CMHS Institutional Review Board.

Name of Members	Institute	Involved in the decision		
		Yes	No (Reason)	
			Absent	Withdrawn from the proceeding
Prof Kato J. Njunwa	UR-CMHS		X	
Prof Jean Bosco Gahutu	UR-CMHS	X		
Dr Brenda Asiimwe-Kateera	UR-CMHS	X		
Prof Ntaganira Joseph	UR-CMHS	X		
Dr Tumusiime K. David	UR-CMHS	X		
Dr Kayonga N. Egide	UR-CMHS	X		
Mr Kanyoni Maurice	UR-CMHS		X	
Prof Munyanshongore Cyprien	UR-CMHS	X		
Mrs Ruzindana Landrine	Kicukiro district		X	
Dr Gishoma Darius	UR-CMHS	X		
Dr Donatilla Mukamana	UR-CMHS	X		
Prof Kyamanywa Patrick	UR-CMHS		X	
Prof Condo Umutesi Jeannine	UR-CMHS		X	
Dr Nyirazinyoye Laetitia	UR-CMHS	X		
Dr Nkeramihigo Emmanuel	UR-CMHS		X	
Sr Maliboli Marie Josee	CHUK	X		
Dr Mudenge Charles	Centre Psycho-Social	X		

After reviewing your protocol during the IRB meeting of where quorum was met and revisions made on the advice of the CMHS IRB submitted on 20th May 2020, **Approval has been granted to your study.**
Please note that approval of the protocol and consent form is valid for **12 months**.

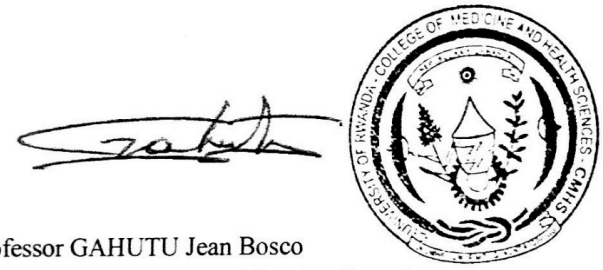
You are responsible for fulfilling the following requirements:

1. Changes, amendments, and addenda to the protocol or consent form must be submitted to the committee for review and approval, prior to activation of the changes.
2. Only approved consent forms are to be used in the enrolment of participants.
3. All consent forms signed by subjects should be retained on file. The IRB may conduct audits of all study records, and consent documentation may be part of such audits.
4. A continuing review application must be submitted to the IRB in a timely fashion and before expiry of this approval
5. Failure to submit a continuing review application will result in termination of the study
6. Notify the IRB committee once the study is finished

Sincerely,

Date of Approval: The 20th May 2020

Expiration date: The 20th May 2021



Professor GAHUTU Jean Bosco
Chairperson Institutional Review Board
University of Rwanda College of Medicine and Health Sciences

Cc:

- Principal College of Medicine and Health Sciences, UR
- University Director of Research and Postgraduate Studies, UR

CENTRE HOSPITALIER UNIVERSITAIRE
UNIVERSITY TEACHING HOSPITAL

Quality Health Care
Training & Research

Ethics Committee / Comité d'éthique

16th Jun,2020

Ref.:EC/CHUK/044/2020

Review Approval Notice

Dear Sylvie Umuganwa,

Your research project: ***"Histological response of Wilms' tumor to preoperative chemotherapy in children aged 0-15 years in children aged 0-15 years in Rwanda. "***

During the meeting of the Ethics Committee of University Teaching Hospital of Kigali (CHUK) that was held on 16th Jun,2020 to evaluate your request for ethical approval of the above mentioned research project, we are pleased to inform you that the Ethics Committee/CHUK has approved your research project.

You are required to present the results of your study to CHUK Ethics Committee before publication by using this link:www.chuk.rw/research/fullreport/?appid=121&&chuk.

PS: Please note that the present approval is valid for 12 months.

Yours sincerely,

Dr Emmanuel Rusingiza Kamanzi
The Chairperson, Ethics Committee,
University Teaching Hospital of Kigali

ETHICS COMMITTEE
CHUK

Scan code to verify.

" University teaching hospital of Kigali Ethics committee operates according to standard operating procedures (Sops) which are updated on an annual basis and in compliance with GCP and Ethics guidelines and regulations "

CENTRE HOSPITALIER UNIVERSITAIRE
UNIVERSITY TEACHING HOSPITAL

CENTRE HOSPITALIER UNIVERSITAIRE DE BUTARE (CHUB)
OFFICE OF DIRECTOR GENERAL

Huye, ...13/07/2020

N° Ref: CHUB/DG/SA/07/1206.../2020

Dr. Sylvie UMUGANWA
Resident in Anatomical Pathology, Y3
School of Medecine and Pharmacy, CMHS, UR
Phone: +250788845615
Email: sylvieumuganwa@gmail.com

Dear Umuganwa,

Re: Your request for data collection

Reference made to your letter requesting for permission to collect the data within University Teaching Hospital of Butare for your research project entitled ***"Histological response of Wils' tumor to preoperative chemotherapy in children aged 0-15 years in Rwanda'."***, based to the approvals **No: 075/CMHS/IRB/2020** from Institution Review Board of University of Rwanda and **No: RC/UTHB/009/2020** from our Research-Ethics Committee, we are pleased to inform you that you are accepted to collect data within University Teaching Hospital of Butare. Please note that your final document will be submitted in our research office.

Sincerely,

Dr. Augustin SENDEGEYA
Director General of CHUB

Cc:

- Ag. Head of Clinical Education and Research Division
- Ag. Director of Research
- Head of Laboratory Department
- Ag. Research officer

CHUB

KING FAISAL HOSPITAL KIGALI

KING FAISAL HOSPITAL, KIGALI
ETHICS RESEARCH COMMITTEE

Patient Centered Care

8th March, 2021

ETHICAL APPROVAL

Dear UMUGANWA SYLVIE

We acknowledge receipt of your study protocol: **"HISTOLOGICAL RESPONSE OF WILMS TUMOUR TO PREOPERATIVE CHEMOTHERAPY IN CHILDREN AGED 0 TO 15 YEARS IN RWANDA."**

After a thorough review, the reviewers of KFH Ethics Research Committee consider this study relevant. The investigator is allowed to start data collection.

N.B.

- The investigator is ***requested to submit one hard copy of his final research results*** in the office of the Directorate of Education, Training and Research at King Faisal Hospital, Kigali

Best Regards

KING FAISAL HOSPITAL-KIGALI

Dr. Dushimiyimana Jean Marie Vianney
Consultant ENT surgeon
Chair, Ethics Research Committee
King Faisal Hospital, Kigali.

CC:

1. Chief Executive Officer_ KFH-Kigali
2. Director of Education, Training & Research_KFH-Kigali
3. Members of the KFH Ethics Research Committee

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, GASABO DISTRICT, P.O. Box 2534 KIGALI, RWANDA