Title:

TREATMENT OF PATIENTS WITH STAGE I FOCAL ANAPLASTIC AND DIFFUSE ANAPLASTIC WILMS TUMOR: A REPORT FROM THE SIOP-WT-2001 GPOH AND UK-CCLG STUDIES

Authors:

William Mifsud[a,b,*], Rhoikos Furtwängler[c], Christian Vokuhl[d], Ellen D'Hooghe[e], Kathy Pritchard-Jones[f], Norbert Graf [c,] Gordan M. Vujanić[a,b,*]

* William Mifsud and Gordan M. Vujanić contributed equally to the paper.

Affiliations:

[a]Department of Pathology, Sidra Medicine, Doha, Qatar

[b]Weill Cornell Medicine - Qatar, Doha, Qatar

[c]Department of Hematology and Oncology, University of Saarland, Homburg, Germany

[d]Department of Pathology, University of Bonn, Germany

[e]Department of Pathology, Oslo University Hospital, Rikshospitalet, Oslo, Norway

[f]Departmental Biology and Cancer Programme, UCL Great Ormond Street of Child Health, University College London, London, UK

Corresponding author:

Gordan M. Vujanić, Department of Pathology, Sidra Medicine, Luqta Street, PO Box 26999, Doha, Qatar, E: gvujanic@sidra.org

Running title:

Outcomes of stage I focal and diffuse anaplastic Wilms tumors

ORCID ID

WM - https://orcid.org/0000-0002-9805-6461

RF - https://orcid.org/0000-0002-1967-8343

CV - https://orcid.org/0000-0002-4138-4536

ED'H - https://orcid.org/0000-0002-0603-8048

KPJ - https://orcid.org/0000-0002-2384-9475

NG - https://orcid.org/0000-0002-2248-323X

GMV - https://orcid.org/0000-0003-0726-6939

Abstract

***Background*:** Anaplasia is an unfavorable prognostic histologic feature in Wilms tumor (WT). Patients with stage I anaplastic WT (AWT) typically achieve good outcomes, albeit with more treatment than for stage I non-anaplastic WT. Since the SIOP-WT-2001 study, patients with focal AWT (FAWT) have been classified as intermediate-risk and received less intense treatment than patients with diffuse AWT (DAWT). The aim of the study was to analyze outcome in these patients.

***Patients and Methods*:** Retrospective analysis of clinico-pathologic features and outcomes of 59 patients with stage I AWT (19 FAWT, 40 DAWT) from the SIOP-WT-2001 GPOH and UK-CCLG groups. The patients with FAWT were treated as intermediate-risk WT, with eight weeks of vincristine and actinomycin D (four weeks pre-operatively, and four weeks post-operatively). For comparison, we also assessed outcomes in 818 patients with stage I intermediate-risk non-anaplastic WT (IR-non-AWT). The patients with DAWT were treated with vincristine, actinomycin D and doxorubicin for 31 weeks. No group received radiotherapy.

***Results*:** Median follow-up was 67.6 months. 4-year EFS and OS in the FAWT group were 87% (95% CI 72-100) and 100%, respectively, in the DAWT group 85% (95% CI 74-98) and 93% (95% CI 85-100), respectively, and in the IR-non-AWT group 91% (95% CI 89-93) and 98% (95% CI 97-99), respectively.

Conclusions: Outcomes for patients with stage I FAWT were comparable with those of other, identically treated, patients with stage I IR-non-AWT. Patients with stage I DAWT

also showed good outcomes, albeit with more intensive chemotherapy than IR-non-AWT, but without radiotherapy.

***Keywords*:** Wilms tumor, anaplasia, stage I, focal and diffuse, outcomes

1. Introduction

Anaplasia in Wilms tumor (WT) was recognized as an unfavorable histologic feature by Beckwith and Palmer [1]. Historically, treatment regimens for anaplastic WT (AWT) have varied in both National Wilms Tumor Study / Children's Oncology Group (NWTS/COG) and International Society of Paediatric Oncology (SIOP) studies, and in most studies, patients with stage I AWT, were shown to have very good outcomes [2-4]. When the original criteria for focal anaplasia (FAWT) and diffuse anaplasia (DAWT) subtyping were revised, FAWT and DAWT were shown to have different prognostic significance in both NWTS and SIOP studies, including in patients with stage I [4,5]. Therefore, in SIOP, since the SIOP-WT-2001 study, patients with stage I FAWT have been classified as intermediate-risk WT and treated identically to patients with intermediate-risk non-anaplastic WT (IR-non-AWT) [6]. Their consolidation treatment, consisting of four weeks of vincristine and actinomycin D (AV) is hence less intense compared to 27 weeks of AV and doxorubicin in stage I DAWT. Importantly, contrary to COG protocols, no radiotherapy is given to patients with stage I AWT [7]. Thus, fewer patients have been at increased risk of developing late sequelae of doxorubicin and radiotherapy.

In this study we analyzed the outcomes of patients with stage I FAWT or DAWT treated according to the SIOP-WT-2001 study regimen, to assess whether the rationale of reduced treatment for patients with stage I FAWT and more intense treatment for patients with stage I DAWT, but without radiotherapy, resulted in good outcomes for patients with both subtypes of stage I AWT.

2. Patients and Methods

2.1. Study population

The cases were identified from the Gesellschaft fur Pädiatrische Onkologie und Hämatologie (GPOH) and the UK Children's Cancer and Leukaemia Group (UK-CCLG) Nephroblastoma Study Group studies and trials (2001-2020). Both study groups participated in the international randomized trial and study, SIOP-WT-2001. The GPOH centers registered patients with renal tumors from 2001-2020 from Germany, Austria and parts of Switzerland. The UK SIOP-WT-2001 Study (2001–2011) registered patients with renal tumors from all pediatric primary cancer treatment centers in the UK and Ireland, and some patients from centers in New Zealand and Australia. The UK Improving Population Outcomes for Renal Tumors of Childhood (IMPORT) study (2012-2020) was a UK multicenter observational study that maintained the SIOP treatment protocol.

Ethical approval for the SIOP/GPOH study was given by the Ärztekammer des Saarlandes (No. 136/01 from 20.09.2002). For the UK-SIOP-WT-2001 study it was given by East Midlands – Derby Research Ethics Committee (National Research Ethics Service (NRES) in the United Kingdom) (reference approval number MREC/01/4/086, from 17.01.2002), and for the IMPORT study the approval was given by the London Bridge REC (reference 12/LO/0101, IRAS ID 62637, from 02.05.2012). The informed consent was provided for all participants. Patients with localized WT were treated as per the SIOP-WT-2001 protocol with preoperative chemotherapy (four weeks with two drugs for

localized tumors), followed by surgery and further chemotherapy for stage I FAWT and DAWT [7-9].

The criteria for inclusion in the study were as follows: a) localized stage I WT, diagnosed in children below 18 years of age; b) diagnosis and stage confirmed by central pathology review (CPR); and c) patients treated according to the SIOP-WT-2001 protocol.

In SIOP-WT-2001 and IMPORT, patients with FAWT were stratified and treated as intermediate-risk WT. Therefore, we used data from all other (i.e., non-anaplastic) intermediate-risk WT (IR-non-AWT) as a baseline comparator group for clinical and outcome analyses.

2.2. Histologic assessment

The diagnosis of anaplasia was based on the presence of three criteria: enlarged, atypical mitotic figures, marked enlargement of the nuclei (at least three-times the size of surrounding nuclei) and associated hyperchromasia [1]. It was further sub-classified into focal or diffuse according to its extent and distribution. *Focal anaplasia* was diagnosed in cases where anaplastic changes were confined to one more clearly defined loci within the primary tumor, without evidence of anaplasia or marked nuclear unrest elsewhere in the tumor, or in extra-renal sites. The diagnosis of focal anaplasia in a tumor with more than one anaplastic focus was acceptable when each focus was small enough to be contained on a single microscopical section, provided that the other criteria for focal anaplasia were met. *Diffuse anaplasia* was defined when any of the following was present: non-localized anaplasia; anaplasia beyond the tumor capsule; anaplastic cells in intrarenal or extrarenal

vessels, renal sinus, extracapsular invasive sites, or metastatic deposits; focal anaplasia with marked nuclear unrest present elsewhere in the tumor; anaplasia not clearly demarcated from non-anaplastic tumor; and anaplasia present in a biopsy or other incomplete tumor sample [5].

Stage I was defined according to the SIOP-WT-2001 Study criteria as a) tumor limited to the kidney and completely excised; b) tumor (pseudo)capsule may be infiltrated, but not penetrated by tumor; c) no viable tumor present in the renal sinus soft tissues or blood/lymphatic vessels; d) intrarenal vessels may contain tumor; e) viable tumor may be present in the perirenal fat but it is covered by a pseudocapsule; f) tumor may show botryoid growth into the renal pelvis or ureter, but does not infiltrate their walls; and g) no open biopsy done [6].

All cases were sampled according to the SIOP-WT-2001 Trial and Study Pathology protocol and submitted for central pathology review for diagnosis, classification, and staging [6,10], performed by the SIOP-UK and SIOP-GPOH Pathology Panel. The sampling of lymph nodes was recorded as “yes” or “no”.

2.3. Treatment

Patients were treated according to the SIOP-WT-2001 Study protocol [7] that included a 4-week preoperative chemotherapy with vincristine (1.5 mg/m^2, weekly) and actinomycin D (45 µg/kg, every 2 weeks) (regimen VA), followed by surgery and, postoperative treatment depended on tumor’s histologic subtype. For patients with FAWT stage I it was another 4 weeks with regimen VA (Vincristine 4 x 1.5mg//m^2, and Actinomycin D 1 x

45µg/kg), and for patients with DAWT stage I, post-operative chemotherapy with regimen VAD continued for further 27 weeks (vincristine 20 x 1.5mg/m2 given weekly day 1 until week 8, and every 3 weeks day 1 and 8, actinomycin D 9 x 45µg/kg every 3 weeks on day 1, doxorubicin 50 mg/m^2, every 6 weeks on day 1, total cumulative dose 250 mg/m^2) [7].

Follow-up information was obtained from the Trial and Study databases containing information regularly received from the participating centers.

2.4. Statistical analysis

Statistical analyses were carried out using the R System for Windows. Follow-up times were calculated from the time of diagnosis. The 4-year event-free survival (EFS) and overall survival (OS) estimates were calculated using the Kaplan-Meier method with 95% confidence interval (95% CI) estimates by the Peto-Peto method. Dichotomous measures were compared using the 2-tailed Fisher exact test. Quantitative variables were compared using the Mann-Whitney *U* test.

3. Results

3.1. Clinical and pathologic characteristics

Out of 194 patients diagnosed with AWT (stages I-V), 59 (30.4%) had stage I, including 19 with FAWT and 40 with DAWT, constituting 2.6% (59/2307) of all WT patients in the study cohort. Their clinical characteristics are summarized in Table 1.

The median age at presentation of patients with stage I AWT was 4.45 years, vs. 2.62 years for patients with stage I non-AWT ($P < 0.001$); the difference in median age vs. patients with stage I non-AWT was significant for patients with stage I DAWT (4.72 years, $P < 0.00001$) but not with FAWT (3.32 years, $P = 0.1$).

3.2. Patient outcomes

The median follow-up time was 67.6 months for survivors with DAWT, 65.9 months for survivors with FAWT, and 62.8 months for survivors with IR-non-AWT. For 19 patients with stage I FAWT, the 4-year EFS estimate was 87% (95% CI 72-100), and the 4-year OS estimate was 100%. For 40 patients with stage I DAWT, the 4-year EFS estimate was 85% (95% CI 74-98), and the 4-year OS estimate was 93% (95% CI 85-100). For 818 patients with stage I IR-non-AWT, the 4-year EFS estimate was 91% (95% CI 89-93) and the 4-year OS estimate was 98% (95% CI 97-99) (Table 2, Figure 1 and Figure 2).

3.3. Patterns of relapses

Relapses occurred in 6/59 (10.2%) patients with stage I AWT, including 2/19 (10.5%) patients with FAWT and 4/40 (10.0%) patients with DAWT. One of the two relapsed patients with FAWT had pre-nephrectomy imaging suggesting regional lymph node

involvement, but regional lymph nodes were not sampled at nephrectomy. Abdominal relapse occurred after 40 months, and the patient was alive 12 months later (at the time of writing). Another patient with FAWT developed a lung relapse 10 months after presentation and was alive three years later. Three patients with DAWT developed lung relapses after 5, 8, and 26 months, respectively. The patient who relapsed after 5 months died after 16 months; the patient who relapsed after 26 months was alive after 152 months. The patient who relapsed after 8 months was lost to follow-up. Another patient with DAWT developed a combined abdominal and lung relapse after 7 months and was alive at the latest follow-up.

4. Discussion

The present study demonstrated that patients with stage I FAWT, treated with vincristine and actinomycin D for four weeks pre-operatively and four weeks post-operatively, showed similar outcomes to other, identically treated, patients with intermediate-risk WT. Patients with stage I DAWT received more intense treatment, consisting of vincristine and actinomycin D over four weeks pre-operatively and 27 weeks of post-operative chemotherapy consisting of vincristine, actinomycin D and doxorubicin, but no radiotherapy. Their outcomes were comparable to those of patients with FAWT and IR-non-AWT (Table 2).

The relative rarity of anaplasia in stage I WT imposes an unavoidable limitation that the two study groups of interest (Stage I FAWT & DAWT) are smaller than the comparator group of Stage I IR-non-AWT, and therefore the absence of a statistically

significant difference in outcome between FAWT, DAWT and IR-non-AWT may be in part related to having relatively few cases of stage I FAWT and DAWT. However, our study included 19 patients with stage I FAWT, i.e., the largest such studied cohort to date, all of whom received identical treatment and were alive at last follow-up (Table 3). As for patients with stage I DAWT, our study of 40 patients (with only one tumor-related death) represents the second largest such cohort, and the only one where all patients received uniform treatment. A comparison to different treatment regimens for stage I AWT used in other studies is presented in Table 3.

In a recent study on stage I DAWT treated with pre-operative and post-operative chemotherapy and no radiotherapy post-operatively, comparable 5-year EFS and OS estimates were obtained; these results indicated that good outcomes can be achieved in stage I DAWT without flank radiotherapy. However, the study included a mixture of patient cohorts with different treatment schedules and no comparison has been made to a non-AWT reference group [11]. In our study, all patients were treated according to the same protocol and without radiotherapy, and we also compared outcomes to a comparator group of intermediate-risk WT on the same protocol.

In the NWTS/COG studies, the treatment of patients with stage I AWT has varied significantly over the last four decades, including the usage of flank radiotherapy [12,13]. The NWTS-3 study (1979-1986) was the first study where patients with AWT were treated differently from non-anaplastic WTs, and it showed that patients with stage I AWT had a comparable outcome to non-AWT (only 1/17 patients with stage I AWT experienced relapse) [2]. In the NWTS-4 study (1986-1994), patients with AWT stage I (both FAWT and DAWT) were treated the same as patients with non-AWT (with vincristine and

actinomycin D only) and showed 85.5% and 93.3% 2-year OS estimate (depending on the actinomycin D administration regimen) [3]. Subsequently, in the NWTS-5 study (1995-2002), patients with stage I FAWT and DAWT were treated with upfront surgery followed by regimen EE-4A, which includes actinomycin and vincristine over 18 weeks and no radiotherapy [12]. However, outcomes were significantly worse in patients with DAWT than in patients with non-AWT/favorable histology WT (Table 2). This led to a substantial increase in treatment intensity of patients with stage I AWT in the subsequent AREN0321 study, consisting of doxorubicin as well as actinomycin D and vincristine over 25 weeks, and 10.8 Gy flank radiotherapy, given to patients with FAWT as well as those with DAWT. The resulting 4-year EFS and OS estimates were 100% (Table 2) [13], albeit in only 18 patients (8 FAWT, 10 DAWT). In order to assess the relative contribution of doxorubicin vs. flank radiotherapy to these excellent outcomes, the authors analyzed outcomes according to therapy in all stage I AWT across NWTS 1-5 (1969-2002) in addition to AREN0321 (2006-2013). They found that doxorubicin, but not radiotherapy, improved EFS in patients with stage I DAWT, without significant difference in OS. There was no significant difference in EFS or OS for patients with either FAWT or DAWT treated with either doxorubicin, radiotherapy, or both. However, as the authors acknowledged, the study covered a very long period of time (1969-2002) during which the definitions of anaplasia and tumor stage, as well as treatment regimes (eight different chemotherapy protocols, varying from 10 weeks to 15 months in duration, with flank radiotherapy used in two protocols) changed, thereby limiting the value of such a comparison [13].

In contrast, in the present study, patients were classified and treated according to the SIOP-WT-2001 protocol [7], in which patients with FAWT were treated as

intermediate-risk WT and patients with DAWT were treated as high-risk WT. We found that patients with stage I FAWT achieved excellent outcomes when treated with vincristine and actinomycin D only for a very short period (4 weeks pre- and 4 weeks post-operatively). Patients with stage I DAWT achieved very good outcomes with 31 weeks treatment (4 weeks pre- and 27 weeks post-operative) with the addition of doxorubicin, and, importantly, without flank radiotherapy.

In the present study 6/59 (10.2%) patients developed relapses, whereas in the AREN0321 and NWTS 1-5 studies relapses occurred in 21/112 (18.8%) patients, and in the 'non-COG' (which included patients from the SIOP-WT-2001 and UKW3 studies) study in 16/95 (17%) patients with DAWT [11,13]. One patient with FAWT stage I in our study who developed a local relapse was suspected to have regional lymph node involvement on pre-nephrectomy imaging, but lymph nodes were not sampled (positive lymph nodes would have made it stage III). Failure to sample lymph nodes may be a factor affecting the risk of relapse, and while we could not test this on the basis of one case, previous studies have shown that patients with WT who had no lymph nodes sampled were more likely to experience relapses [14,15]. Most relapses developed early, as in other studies [11], and the two patients who died both relapsed before 6 months after diagnosis suggesting treatment resistance.

In summary, we showed that outcomes for patients with stage I FAWT were comparable with those of other, identically treated, patients with stage I IR-non-AWT. Patients with stage I DAWT showed good outcomes, albeit with more intensive chemotherapy than IR-non-AWT, but without radiotherapy.

Author contributions

Gordan M. Vujanić conceived the study, reviewed the cases included in the study, performed analyses, wrote the article, and reviewed and edited the final article drafts. **William Mifsud** conceived the study, performed the analyses, co-wrote the article, and reviewed and edited the final article drafts. **Rhoikos Furtwängler** provided the clinical data for the study, reviewed and edited the final article drafts. **Christian Vokuhl** reviewed the pathology of cases included in the study, reviewed and edited the final article drafts. **Ellen D'Hooghe** sorted out the data included, reviewed and edited the final article drafts. **Kathy Pritchard-Jones** provided the clinical data for the study, reviewed and edited the final article drafts. **Norbert Graf** provided the clinical data for the study, reviewed and edited the final article drafts.

Ethics Statement

Ethical approval for the UK-CCLG-SIOP-WT-2001 study was given by East Midlands – Derby Research Ethics Committee (National Research Ethics Service (NRES) in the UK) (reference approval number MREC/01/4/086, from 17.01.2002). For the IMPORT study the approval was given by the London Bridge REC (reference 12/LO/0101, IRAS ID 62637, from 02.05.2012), and for the SIOP/GPOH study by the Ärztekammer des Saarlandes (No. 136/01 from 20.09.2002). Informed consent was provided for all participants.

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Disclosure

The authors have declared no conflict of interest.

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Figure Legends

Figure 1. Estimated, (A) event-free survival for patients with stage I diffuse anaplasia, focal anaplasia, and intermediate-risk non-anaplastic Wilms tumor, (B) overall survival for patients with stage I diffuse anaplasia, focal anaplasia, and intermediate-risk non-anaplastic Wilms tumor.