Title:

The effect of pre-operative chemotherapy on histological sub-typing and staging of Wilms tumours: the United Kingdom Children's Cancer Study Group (UKCCSG) Wilms tumour trial 3 (UKW3) experience

Authors

Gordan M. Vujanić[1][*][#], Ellen D'Hooghe[2][*], Sergey D. Popov[3], Neil J. Sebire[4], Anna Kelsey[5]

*Equal first authors

corresponding author

Affiliations:

[1]Department of Pathology, Sidra Medicine, Doha, Qatar, T: +974 4003 2912, E-mail: gvujanic@sidra.org

[2]Department of Pathology, Oslo University Hospital, Rikshospitalet, P.O. Box 4950 Nydalen, 0424 Oslo, Norway, T: +47 2307 1400, E: eldhoo@ous-hf.no

[3]Department of Cellular Pathology, University Hospital of Wales, Cardiff, CF14 4XN, U.K., T: +44 29 2074 2703, E-mail: sergey.popov@wales.nhs.uk

[4]Department of Histopathology, Great Ormond Street Hospital for Children NHS Trust, Great Ormond Street, London, WC1N 3JH, U.K., T: +44 20 7829 8663, E-mail: sebirn@gosh.nhs.uk

[5]Department of Diagnostic Paediatric Histopathology, Royal Manchester Children's Hospital, Oxford Road, Manchester, M13 9WL, U.K., T: +44 161 701 2247, E-mail: anna.kelsey@cmft.nhs.uk

Address for correspondence:

Gordan M. Vujanić, Department of Pathology, Sidra Medicine, Sidra Hospital H2M Pathology Room 2MF 140, Al Luqta Street, Doha, Qatar, T: +974 4003 2912, F: +974 4003 3066, E: gvujanic@sidra.org

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Abbreviations	Full names
UKCCSG	United Kingdom Children's Cancer Study Group
UKW3	United Kingdom Wilms Tumour Trial 3
WT	Wilms tumour
IS	Immediate surgery
PCT	Pre-operative chemotherapy
NWTSG	National Wilms Tumor Study Group
SIOP	International Society of Paediatric Oncology
COG	Childern's Oncology Group
GPOH	German Paediatric Oncology and Haematology

Abstract

Background: Two principal approaches to Wilms' tumour (WT) treatment are immediate surgery (IS), and pre-operative chemotherapy (PCT), and both treatments use risk-adapted approach that includes histological sub-classification of the tumour, combined with additional prognostic factors. In the UKW3 trial these two approaches were compared. The aim of the present study was to compare histological features between the two groups, to assess impact of PCT on distribution of histological sub-typing and staging, and to evaluate whether PCT resulted in more staging discrepancies between local and central pathology review (CPR).

Material and Method. The cases were identified from the UKW3 Trial database. The criteria for inclusion in the study were: unilateral, non-metastatic, non-anaplastic WTs, and submitted for CPR with an adequate number of slides. They were sub-classified according to the NWTS and later the SIOP 9301 criteria.

Results. There were 244 WTs in the IS and 182 in the PCT group sub-classified as follows: blastemal 86 (35%) vs. 9 (5%), epithelial 34 (14%) vs. 12 (7%), stromal 12 (5%) vs. 25 (14%), mixed 112 (46%) vs. 45 (25%), respectively, plus 40% regressive and 10% completely necrotic WTs in the PCT group. The differences between the two groups for blastemal and mixed types were statistically significant. In the PCT group there was a significant decrease in stage III tumours. The discrepancies in staging between local and CPR were not significant.

Conclusion: PCT significantly altered histological features and typing of WTs. It resulted in fewer stage III tumours, and staging discrepancies were equally represented in both groups.

INTRODUCTION

Nephroblastoma or Wilms tumour (WT) is the most common renal tumour of childhood. Its prognosis has significantly improved over the last decades and its cure rate is now exceeding 90% in patients with localised, non-anaplastic WT, and overall long-term survival for all types and stages of over 80%.[1] There are two standard approaches to WT treatment: the International Society of Paediatric Oncology (SIOP) use pre-operative chemotherapy (PCT),[2,3,4] and the Children's Oncology Group (COG) (formerly National Wilms Tumor Study Group - NWTSG) use immediate surgery(IS) as the first step in treatment.[4,5] Both groups use post-operative chemotherapy, except for selected cases not receiving adjuvant therapies, and in higher stages radiotherapy in a risk-adapted approach.

WT is composed of three components, blastemal, epithelial and stromal, which can be present in different proportions, and may show varying degrees and lines of differentiation, resulting in numerouspatterns. PCT may result in necrosis or regressive changes of tumours, and can also alter the histological features of all components by further differentiation and maturation (Fig. 1A).[6,7,8] Typical chemotherapy-induced changes consist of a coagulative-type necrosis with no recognisable structures, and/or necrosis of blastemal cell or neoplastic tubules presenting as 'ghost' cells with loss of nuclei and cytoplasmic details, fibromyxoid, hypocellular stroma with variable amount of foamy and/or haemosiderin-laden macrophages, dystrophic calcification, granulation and inflammation.

The subtyping of WT is based on histological features and is a part of the risk assessment in the treatment protocols. In the SIOP Working Classification tumours are divided into three risk groups, low, intermediate, and high risk tumours. Completely necrotic type is the only pre-treated WT in the low risk group, blastemal type and diffuse anaplasia representing the high risk group, and epithelial, stromal, mixed, regressive type, together with focal

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anaplasia being the intermediate risk group.[9] In the COG classification, only favourable (non-anaplastic) and unfavourable (anaplastic) histology WTs are recognised.[4] However, even in the COG when preoperative chemotherapy is given (inoperable and bilateral cases), blastemal type is regarded as a bad prognostic factor.[10]

The advantage of the COG approach, from a pathology point of view, is that the tumour's histological features are unaffected by therapy, and hence sub-typing and staging are simpler. Using the SIOP protocol, pathologists are assessing tumours after PCT, which both alters histological features and may make sub-typing and staging more challenging. Histological typing and staging remain the most important parameters determining post-operative therapy and prognosis, despite recent introduction of molecular markers in the treatment stratification.[4]

No previous systematic study has been done on comparison of histological features of un-treated and pre-treated WTs, but there were rare non-systematic studies which partly addressed this issue.[11,12] The aim of this study was to compare histological features in nephrectomy specimens between primarilyoperatedWTs and WTs treated with pre-operative chemotherapyin the UK Children's Cancer and Leukaemia Group (CCLG) Wilms' Tumour Trial 3 (UKW3), and to assess the impact of therapy on distribution of histological sub-typing and staging. In addition, we evaluated whether pre-operative chemotherapy resulted in more difficulties in staging of tumours due to the presence of chemotherapy-induced changes.

MATERIALS AND METHODS

All children up to the age of 16 years with a suspected renal tumour referred to any of the 25 children's cancer treatment centres (22 UKCCLG centres, plus Oslo, Norway, and Adelaide and Sydney, Australia) were eligible for registration in the UKW3 trial which ran from October

1991 to March 2001. The purpose of UKW3 trial was to determine whether patients receiving preoperative chemotherapy with vincristine and actinomycin D for non-metastatic WT have a more advantageous stage distributionand so need less treatment compared to patients who have immediate nephrectomy, without adversely affecting outcome.[3] It also included routine percutaneous cutting needle biopsy at diagnosis for all tumours receiving pre-operative chemotherapy without upstaging the tumour. The inclusion criteria for the present study were: a) unilateral WTs; b) non-metastatic; c) non-anaplastic; and d) submitted for central pathology review with an adequate number of slides (>5, 70% of cases had >10 slides, median 14) and reviewed by the CCLG Pathology Panel. Although at the present time extensive sampling is recommended, in previous studies a minimum of 5 slides was regarded as sufficient.[13,14] The pre-operative chemotherapy for unilateral, non-metastatic WTs consisted of six weeks of treatment with vincristine and actinomycin D.

The tumours were originally sub-classified according to the NWTS criteria which did not take into account chemotherapy-induced changes, but only recognised two types: non-anaplastic and anaplastic WTs. Later, for the purpose of this study, all tumours treated with pre-operative chemotherapy were re-classified according to the SIOP 93-01 criteria as follows: if more than 1/3 of the tumour was viable, and if more than 2/3 of the viable tumour consisted of a certain component, the tumour was designed accordingly (blastemal type, epithelial type, stromal type, regressive type), if no component was predominant, the tumour was regarded as mixed. If no viable tumour was identified, the tumour was classified as completely necrotic. Anaplastic WTs were sub-classified as focal and diffuse anaplasia.

Originally, tumours in both IS and PCT groups were staged according to the NWTS4 criteria[1] but later, tumours treated with PCT were re-staged according to the SIOP 93-01 staging criteria.[16]

The significance in differences in proportions of cases by histological type and stage between groups were evaluated using comparison of proportion (Chi squared) test with $P<0.05$ regarded as statistically significant. For comparison of proportions of types, Fisher exact test was used[17].

RESULTS

In total, 718 cases of WTs were enrolled in the UKW3 trial. In six WT cases no nephrectomy was performed. In 63 WT cases the diagnosis of non-anaplastic WT was confirmed either on biopsy or nephrectomy, but the material was regarded as inadequate for further sub-classification as they were submitted with 1-5 slides only. Bilateral (53 cases) and metastatic (116 cases) WTs were excluded since they received different pre-operative treatment. In addition, there were 36 unilateral, non-metastatic anaplastic WTs including 17 in IS group and 19 in PCT group. Focal anaplasia was found in 4/17 (24%) of primarily operated, and in 8/19 (426%) of anaplastic WTs treated with pre-operative chemotherapy. Diffuse anaplasia was found in 13/17 (76%) of primarily operated, and in 11/19 (58%) of anaplastic WTs treated with pre-operative chemotherapy. Anaplastic WTs were excluded since they are regarded as more chemotherapy resistant and therefore not comparable for analysis done on non-anaplastic WTs.

The present study group comprised 426 patients which fulfilled the criteria for inclusion in total, including 244 WTs in the IS and 182 WTs in the PCT group. There were 11 patients younger than 6 months of age in the IS group, and only one patient in the PCT group (treated with pre-operative chemotherapy because tumour was regarded as inoperable).

The results of distribution of WTs types are presented in Table 1 and Table 2.

There was a marked significance in the proportion of blastemal and mixed types in the randomised cases between the groups (Table 1), and in each histological type when both randomised and non-randomised cases were analysed together (Table 2).

In the IS group (all cases together), the most common type was mixed 112 (46%) followed by blastemal 86 (35%) WTs. In the PCT group, the most common type was regressive 72 (40%) followed by mixed type 45 (25%), whereas blastemal type was the least common - 9 (5%) cases.

The stage distribution was, however, similar. In both groups of randomised cases, over 50% of cases were stage I (Table 3), and it was only stage III in which the difference was statistically significant (*P*=0.009). Similarly, when both randomised and non-randomised cases were analysed together (Table 4), the only statistically significant difference was in the stage III tumours (*P=0.047*).

In 78/426 (18%) cases there was a discrepancy between the local pathologist and central pathology review regarding staging of tumours (Table 5), including 42/244 (17%) in the IS group and 36/182 (20%) in the PCT group. In both groups, cases were under- and over-staged, and the biggest problem seemed to be distinguishing between stage I and stage II, with 55% tumours (in both groups together) being under-staged, and 61% over-staged by the local pathologists. However, these staging errors were not statistically significant between the groups.

DISCUSSION

Pre-operative chemotherapy has been used in the SIOP trials and studies since mid-1970-ies, with the aims of minimising the risk of tumour rupture, inducing a more favourable stage distribution, and to allow histological response to chemotherapy to be evaluated in risk

stratification of post-operative treatment.[2-5,18] However, the histological assessment of tumours after chemotherapy makes sub-typing and staging more challenging.[7,8] In some WTs it may be difficult to distinguish viable, hypocellular stroma from chemotherapy-induced changes, and some post-chemotherapy histological changes may mimic anaplasia.[19] The histological features of viable tumour can be altered as chemotherapy is known to induce differentiation and maturation in WT,[7,8] as well as in other paediatric tumours, such as neuroblastoma[6,20] and rhabdomyosarcoma.[6,21] Nevertheless, standard pre-operative chemotherapy makes it possible to identify prognostically important WT types, such as completely necrotic and blastemal types, and it does not obscure or induce anaplasia.[12,18,22] Nephrogenic rests are often preserved after standard pre-operative chemotherapy, which is a clue to the diagnosis of WT in a completely necrotic tumour.[13,23] Pre-operative chemotherapy does require a more complicated staging system but it also downstages tumours, resulting in less post-operative treatment.[14,24]

In addition to bilateral and metastatic tumours which were excluded because their pre-operative treatment is longer and more aggressive, anaplastic WTs were also excluded from detailed analysis because they are regarded as chemotherapy-resistant and this group was not included in other similar studies with which we compared our results.[11,12]

Our study is unique because it included two groups which are not usually covered by other studies. The only similar study is the third Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP) Wilms Tumor 2003 study, but it did not include a study on comparison of IS and PCT groups.[24] The present study included two groups which had a comparable and a sufficient number of patients, in addition to a group representative of the general WT population. In the previously published studies that compared WTs treated with IS *vs* PCT, the groups were unequally distributed in terms of age and clinical features. In the German Paediatric Oncology and Haematology (GPOH) study,[11] there were 71 cases in the IS group and 258 in the PCT group. The IS group was very mixed and included 14 patients under 6

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months of age, three over 16 years of age, 28 patients with doubt in diagnosis, three patients with emergency indications, 15 patients who had IS because a surgeon was ignorant of the trial, and 3 patients for other reasons.[11] In the American studies on the effect of pre-operative chemotherapy on the different subtypes of WT,[8,12] the cases which were included in the pre-operative chemotherapy group were highly selected by being deemed inoperable because of large size or extent of disease, thus preferentially including higher stage tumours. In addition, pre-operative chemotherapy regiments were not standardised regarding type of chemotherapy used, the dose or duration of pre-operative treatment. Some patients even received pre-operative radiotherapy. In Zuppan *et al.*'s NWTS-3 series the histological features of the 83 WTs treated with pre-operative chemotherapy were compared to pre-chemotherapy biopsies, which were only available in 36 cases. In addition, only 39 WTs were non-metastatic (stages I-III).[12] Guarda *et al.*'s study comprised 21 primarily unresectable cases treated with pre-operative chemotherapy, that were compared to 20 cases from a control IS group which had small tumours, and they included no histological sub-typing.[8]

Our results, both in the randomised cases only and when randomised and non-randomised cases were analysed together, based on large numbers of broadly comparable cases representative of the overall WT population, confirm that pre-operative chemotherapy significantly alters histological features and sub-typing of WTs, similar to the results of other studies (Table 6).[11,12,15,26]

The tumour response to pre-operative chemotherapy is of prognostic significance[11,13] and is therefore taken into account when sub-classifying WTs.[9] Many WTs respond well to pre-operative chemotherapy, resulting in massive necrosis and shrinkage, and completely necrotic WTs have an excellent prognosis, and are classified as low risk.[11] In the present study, 50% of WTs showed marked chemotherapy-induced changes (regressive and completely necrotic types), with 10% being completely necrotic. Similar results were obtained from the SIOP-

9/GPOH and NWTS3 studies (Table 6) in which 43% and 39% of cases, respectively, showed extensive or complete necrosis.[11,12] In the analysis of WT sub-types from the SIOP 93-01 trial, WTs with >90% necrosis had better prognosis than other WT sub-types from the intermediate risk group.[26] With a significant decrease in the percentage of blastemal sub-type in the PCT group, it is evident that the blastemal component is most chemotherapy sensitive WT component. However, some WTs failed to show significant response, which was of prognostic significance for those of blastemal subtype (when blastema comprises >2/3 of the viable non-regressive tumour), since they were associated with a poorer outcome,[11] and are now regarded as a high risk tumour group requiring more intensive post-operative treatment.[1,9]

There was a noticeable difference in the proportion of stromal type WT between the two groups in the present study when randomised and non-randomised cases were analysed together, with only 5% in the IS group vs. 14% in the PCT group. This is likely to be for two reasons: firstly, because the stromal component is more chemotherapy-resistant, and secondly, because chemotherapy itself may induce stromal differentiation. This could also partly explain the lower percentage of mixed type WTs. In WTs treated with pre-operative chemotherapy the stromal component often shows rhabdomyoblastic differentiation and tumours may show further growth or no shrinkage at all.[27] The lower proportion of epithelial WT in the PCT group (randomised and non-randomised cases together) may be explained by good response of early epithelial structures to chemotherapy, whereas more differentiated epithelial elements are resistant.[21,29] The numbers in the randomised cases only were very low and did not reach statistical difference. Although all WT components may be chemotherapy resistant, epithelial and stromal types have an excellent prognosis in lower stages.[16,22] Therefore, and on the basis of the GPOH studies[30] regarding tumour volume, epithelial and stromal types are now excluded from the group of WT tumours which in the UMBRELLA 2016 study will be treated more

aggressively in the UMBRELLA 2016 study if the volume is more than 500ml after pre-operative chemotherapy.[4,31]

Studies comparing histological features of WTs in pre-chemotherapy biopsies and post-chemotherapy nephrectomies have shown very similar results, although no sub-typing was possible in the biopsy group.[29,32] In Vujanic *et al.*'s study, 89% of biopsied tumours showed at least some blastema, whereas at nephrectomy only 50% of tumours contain some blastema.[29] In Taskinen *et al.*'s series the proportions of the blastemal, stromal and epithelial components were 55%, 28% and 2%, respectively, in the biopsy samples, and 5%, 15% and 15%, respectively, in the nephrectomy specimens (*P*-values 0.002, 0.599 and 0.005, respectively).[32]

The present study showed similar results in the stage distribution as the randomised UKW3 trial, where stage I was found in 54.3% vs. 65.2%, stage II in 14.9% vs. 23.9%, and stage III in 29.8 vs. 9.8% of IS vs PCT group, respectively. Only distribution of stage III between the two groups was statistically significant.[3] in the present study, the only statistically significant difference was observed in stage III (*P=0.009*). A likely explanation why there was no increase in a number of stage I and II tumours is that in the UKW3 trial, 38% of patients did not meet the inclusion criteria for randomisation (including metastatic, bilateral and 'inoperable' cases, and infants aged <6 months), and 38% were further not randomised because of clinical or parental decision.[3,16] As a result, there was a bias in assigning patients with a higher clinical stage into the PCT group.[15] The SIOP 9/GPOH study showed 44% vs 61% stage I, 17% vs. 23% stage II, and 39% vs. 16% stage III patients in the IS vs. PCT groups, respectively.[10] On the other hand, the NWTS 5 study showed fewer lower stage WTs: stage I – 28.5% (415 patients), stage II 38% (555 patients), and stage III 33.5% (488 patients).[32]

Although staging of WTs treated with pre-operative chemotherapy is often challenging, the findings of the present study showed no significant differences in discrepancy rates between the institutional and review pathologists in two group (17% and 20%, respectively). Central

pathology review in SIOP trials and studies also reported differences in stage in 9-14% of cases, even when only a small number of slides were available for assessment.[3,34,35] Equally, in the NWTS 5 trial, there were stage discrepancies in 19% of anaplastic WTs.[36] These data demonstrate that staging in itself may be challenging, not only in pre-treated cases, where chemotherapy-induced changes make staging more complicated, but also in primarily operated WTs. Therefore, rapid central pathology review is necessary to ensure that patients are treated according to accurate tumour type and stage.[34]

CONCLUSION

The present study has shown that pre-operative chemotherapy has a significant impact on the histological appearance of WTs. The response to pre-operative chemotherapy can be regression, further differentiation and maturation, or resistance. Around half of WTs treated with pre-operative chemotherapy show either complete or predominant necrosis which results in percentage reduced proportion of blastemal and mixed type WT. The risk stratification is based on semi-quantification of these responses and viable tumour components, and is important for post-operative treatment intensity in the SIOP protocol. We confirmed the result of the randomised UKW3 trial that pre-operative chemotherapy results in a significant reduction in stage III tumours. Finally, there is no significant difference in staging discrepancy between the institutional pathologists and central pathology reviewers between groups, indicating that staging is equally challenging in WTs treated with primary surgery and those treated with pre-operative chemotherapy.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ACKNOWLEDGMENT

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