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# An analysis of histology and DNA-ploidy in primary wilms tumors and their metastases and a study of the morphological effects of therapy *

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**Summary.** In children with Wilms' tumours the length of survival is greatly influenced by success in preventing or controlling metastatic disease. The current study focuses on the morphological aspects of metastases when compared with the primary tumour. In 8 patients it appeared that blastema is the most likely component to metastasize, whereas epithelial and stromal components were hardly, if at all, represented in metastases. Furthermore, flow cytometric DNA ploidy determinations on 4 cases showed that both the primary tumours and the metastases had stemlines in the diploid and low aneuploid (hyperdiploid) range. Finally, in four cases the influence of therapy on morphology of the primary tumours was analyzed. In these cases blastema seemed to be the component most sensitive to therapy. Thus, blastema seems to play a central role in prognosis of Wilms' tumours; either reacting to therapy or, if insensitive, by metastasizing.

**Key words:** Wilm's tumor – Chemotherapy – DNA-flowcytometry – Blastema – Metastasis

## Introduction

Wilms' tumour or nephroblastoma is the most frequent renal malignancy in children (Breslow and Langholz 1983; Harms et al. 1983; Kramer et al. 1983) and constitutes about 5 percent of all pediatric tumors (Breslow and Langholz 1983; Kramer et al. 1983; Jones 1978). After the introduction of combined surgical, radio-, and chemotherapy, the 5-year survival has risen to 80% (Jones 1978; Becht et al. 1983; Green and Jaffe 1978). One of the causes of death is failure to control metastatic disease (Mohr and Murphy 1974) either present at the time of diagnosis or developing during the course of the illness. Despite the importance of metastases, little attention has been paid to their morphological characteristics.

In the current study a morphological analysis of both primary tumours and their metastases was made in 8 cases. In all material a semi-quantitative analysis was made of the constituent components i.e. blastematous, epithelial, and stromal components. In four of these cases DNA-ploidy was determined both in the primary tumours and in their metastases. Finally in order to study the influence of chemotherapy on Wilms' tumours, primary tumour material obtained before and after treatment was carefully analyzed in four cases.

## Materials and methods

Of a group of 32 patients with Wilms' tumours diagnosed in the university of Groningen between 1971 and 1983, 4 presented with metastatic disease and 10 developed metastases during the course of the illness. In 8 of these cases initial or late metastases were available for histological evaluation and for comparison with the primary tumour (Table 1). All of these patients were treated with a combination of nephrectomy and chemotherapy (vincristin and actinomycin D) and all but patient 3 received radiation therapy. Patient 2 also received adriamycin. In patient 1–3 a nephrectomy was performed before any treatment was given. In patients 4 and 5 with bulky disease, and in patient 6–8, diagnosed in the first period of the study, radiotherapy and chemotherapy were started before nephrectomy.

All available haematoxylin and eosin stained sections of these cases were reviewed and all but one appeared to be of standard-risk histology (Beckwith 1983; Beckwith and Palmer 1978; Schmidt and Harms 1983). In patient 2 an anaplastic rhabdomyosarcomatous component was observed (for details: Molenaar et al., submitted). The sections were analyzed for the

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**Table 1.** Patient DATA

| Patient | M/F | Age (months) | Stage | Meta-stases* | Haematogenous/ lymphogenous |
|---|---|---|---|---|---|
| 1 | F | 15 | III | 0 | L |
| 2 | F | 110 | III | 0 | L |
| 3 | F | 75 | I | 3 | H[b,d] |
| 4 | F | 53 | V | 3 | H[b,c] |
| 5 | F | 59 | III | 0 | L |
| 6 | F | 49 | III | 8 | H[b,c] |
| 7 | M | 47 | II | $6\frac{1}{2}$ | H[b] |
| 8 | F | 44 | IV | 0 | H[b] |
| 9 | M | 14 | V | – | – |
| 10 | F | 50 | V | – | – |

[a] Time in months between diagnosis of the primary tumor and development of the metastasis
[b] Lung metastasis
[c] Liver metastasis

presence of blastematous, epithelial and stromal components as defined by Bennington and Beckwith (1975). Only clear cut tubular formations with a central lumen were considered to be epithelial. In the stromal component the presence of rhabdomyoblastic features was specifically mentioned. A quantification of the different components was made by means of point-counting as described by Pfeiffer (1980). Briefly, a grid displaying curved lines with 18 regularly spaced cross-marks was fixed in the ocular (Fig. 1) and the components covered by cross-marks were scored. In a test series the adequacy of sampling was determined by plotting an average summation graph: the mean was calculated after each field. It appeared that after counting of 10 fields in each section the mean became steady (Anderson 1982). Therefore, 10 fields per section were measured in the cases studied. Moreover, from each patient at least 2 sections of the primary tumour were measured, resulting in a total of 23 sections in the 8 patients and for at least one of the metastases resulting in a total of 20 sections. After completion of the counting, non relevant structures such as blood vessels were subtracted and the percentage of each component was calculated (Fig. 2). The reproducibility of the counts was tested and appeared to vary between 5 and 10%.

For flow cytometry suspensions of single nuclei were made from frozen material from both the primary tumours and the metastases of patients 2 and 3 and from the primary tumour of patient 1 and stained with propidium iodide (Vindeløv et al. 1982; Vindeløv et al. 1983a). The samples were measured on an ICP-22 flow cytometer using trout red blood cells (TRBC) as internal ploidy standard (Vindeløv et al. 1983a, 1983b). Since normal human cells in the $G_0$. $G_1$ phase of the cell cycle have a DNA content 1.24 times that of TRBC, cell populations with deviating modal DNA contents may be considered aneuploid and the DNA-index (DI) as compared to normal cells may be calculated. From patient 4 and the metastasis of patient 1 only paraffin embedded material was available. Nuclear suspensions were made of 30 micro sections of this material and stained with 4, 6-diamino-2-phenylindole (DAPI) (Hedley et al. 1983). Since no internal ploidy standard can be used with this material DI's have to be derived from comparison between assumed normal and tumour peaks in the DNA profile.

In cases 4 and 5 nephrectomy was performed after chemo- and radiotherapy, where a biopsy (case 4) and a debulking procedure (case 5) were performed before therapy. In another case (patient 9) with multiple bilateral tumours but without metastatic disease, bilateral biopsies were taken. After chemotherapy a right-sided nephrectomy was performed and biopsies were taken from the left side. In the histological sections of these three cases the viable and necrotic components in the tumour after treatment were compared morphologically. In an additional case (patient 10) the tumour specimen obtained after 4 days of vincristin treatment contained both Wilms' tumor and nephroblastomatosis. The morphological effect of chemotherapy on the different components was studied.

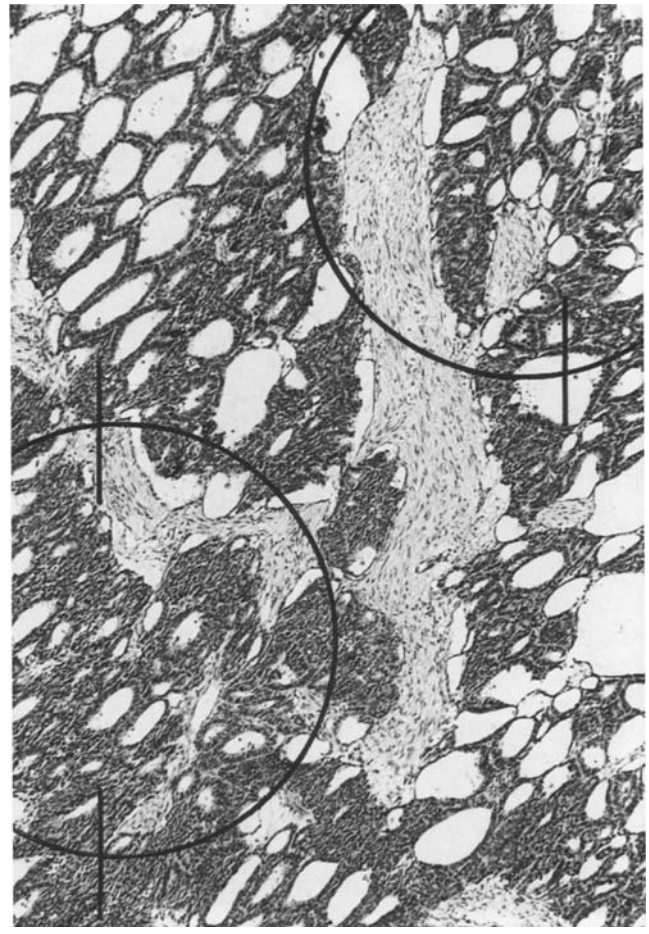


**Fig. 1.** Grid consisting of curved lines with cross-marks. The different components covered by the cross-marks were counted. (H&E, ×56)

## Results

Results of semi-quantitative analyses are represented in Fig. 2. In cases 5 and 8 the metastases were entirely necrotic. In 5 of the remaining 6 cases the blastematous component was relatively more heavily represented than in the primary tumour (Fig. 3). In the sixth case (case 6) the blastematous component in the metastasis comprised 68 percent of counted points and was 79% in the primary tumour. However, 23% of the metastasis appeared necrotic compared with 2% in the primary tumour, which means that of the viable tumour compo-

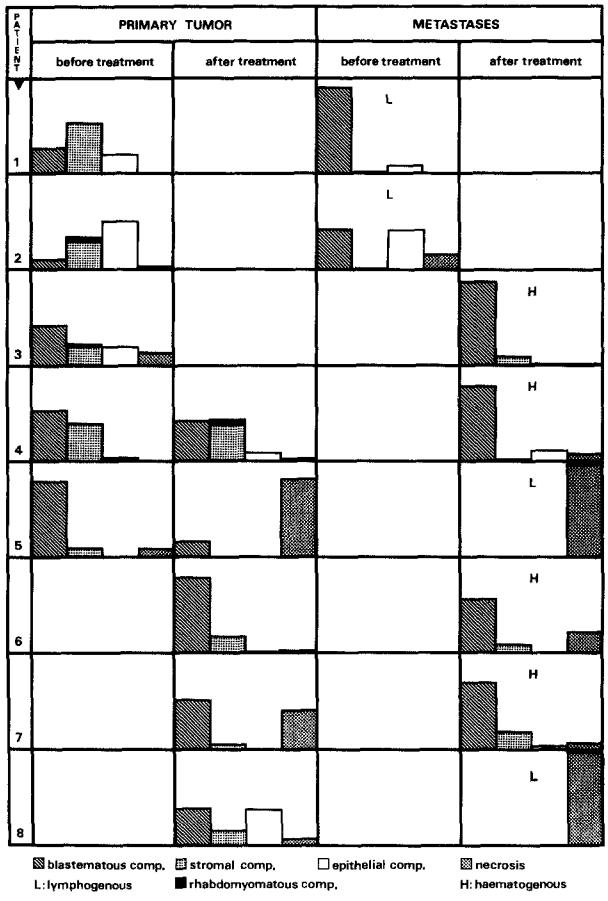



**Fig. 2.** Graphical representation of percentages of each tumour component analysed in each of the 8 patients. The relative contribution of each component is represented as percentage of the total tumour tissue (cf Material and methods)

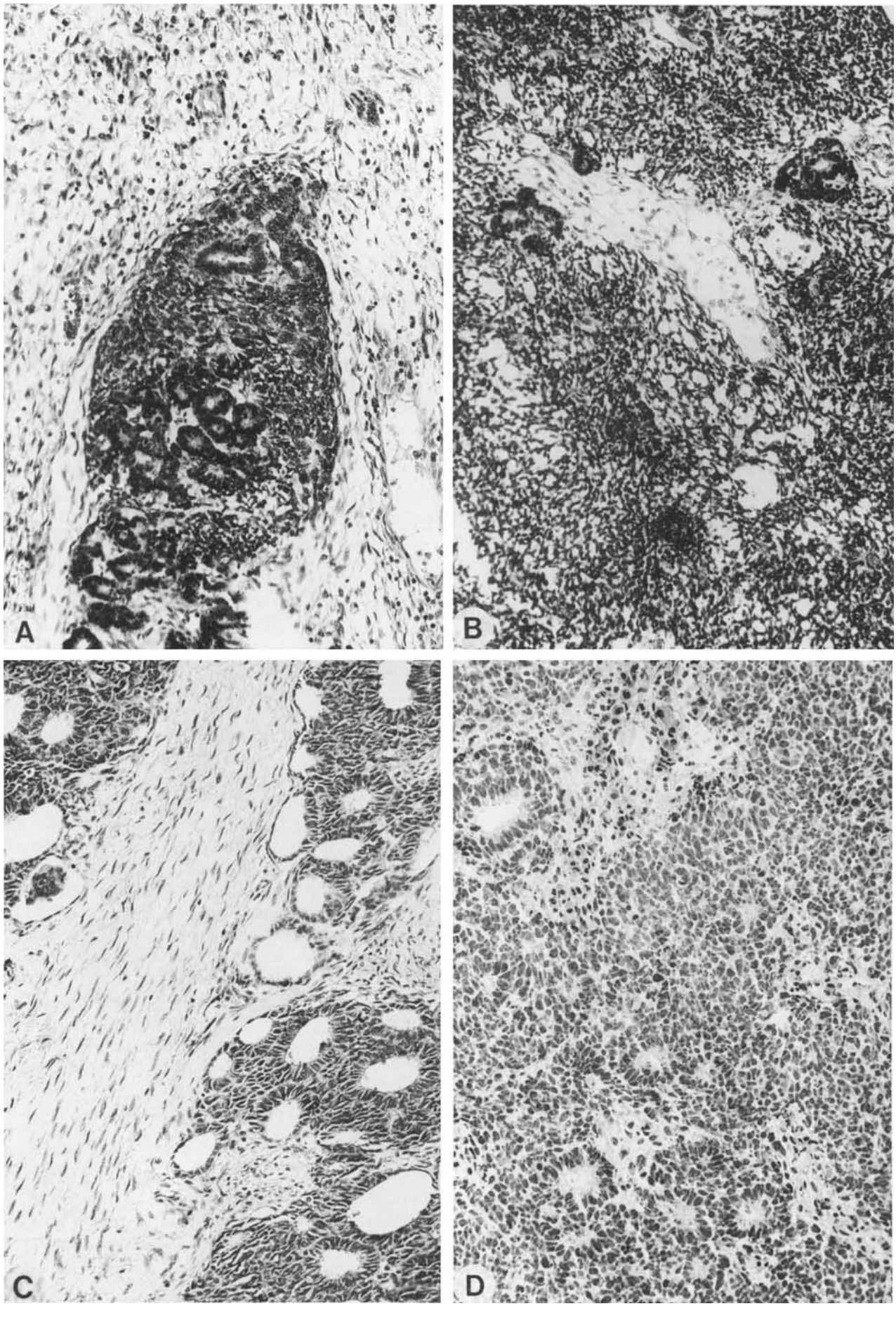
nents, blastema was more heavily represented in the metastasis than in the primary tumour. In respect to blastema, no differences were found between lymphogenous and haematogenous metastases or between pretreated and non-pretreated primary tumours. In both lymphogenous metastases with viable tumour (cases 1 and 2), the epithelial component was less heavily represented than in the primary tumour (Fig. 3A, B). In the four haematogenous metastases, the epithelial component was less represented than in the primary tumour in cases 3 and 6, but more heavily represented in cases 4 and 7 (Fig. 3C, D). In case 7, no epithelial component was found in the primary tumour, but was present in the metastasis. The stromal component barely or not represented in the lymphogenous metastases. However in the four hematogenous metastases, a stromal component was observed, but was relatively less represented than in the primary tumour.

The DNA profiles of the cases measured are represented in Fig. 4. In 2 primary tumours in which DI's could be calculated by comparison with TRBC's (patients 2 and 3), DI's were in the hyperdiploid range. Second, smaller peaks, were observed in the hypertetraploid range, corresponding to tumour cells in the G2M-phase of the cell cycle. In patient 3 the metastasis revealed a similar pattern as the primary tumour, but in patient 2 the metastasis was diploid. Since the peak in the tetraploid range represents cell populations in an actively reproducing phase, this peak is more likely to be derived from tumour cells than from normal cells. In case 1 the primary tumour revealed a diploid DNA profile, whereas only paraffin material was available from the metastasis. Since tumour cells greatly outnumbered normal cells, it may be assumed that the higher peak in the $G_1$-area is derived from tumour cells resulting in a DI of 1.13. In case 4 the DNA profiles are very poor, presumably due to insufficient fixation. It is tempting to assume that in the primary tumour, as in the other cases, the second peak is derived from the tumour cells. In that case the DI is 1.16. In the metastasis separate peaks can hardly be distinguished. A DI of approximately 1.18 is suggestive.

In cases 4, 5 and 9 material from the primary tumour was available before and after treatment. In all three cases the blastematous component was distinctly less prominent after treatment than before (Fig. 2). In case 4 the stromal component was roughly similar and the epithelial component relatively increased. In case 5 the stromal component was not observed anymore and the epithelial component was present neither before nor after treatment. In case 9 the biopsies from both sides revealed a triphasic nephroblastoma with clearcut blastematous areas, whereas neither in the post-treatment biopsy of the left side, nor in the extensively sampled nephrectomy specimen could blastema be found. The tumour tissue revealed highly differentiated viable areas next to entirely necrotic areas. In case 10 the primary tumour was studied after 4 days of chemotherapy. In this case intact epithelial and stromal components were found within necrotic areas. In the latter areas a blastematous character could often be recognized (Fig. 5A). Adjoining nephroblastomatosis areas appeared entirely unaffected (Fig. 5B).

## Discussion

This study examined the morphological aspects of metastatic disease in Wilms' tumours. Unfortunately, the number of cases is relatively small and


A
B
C
D

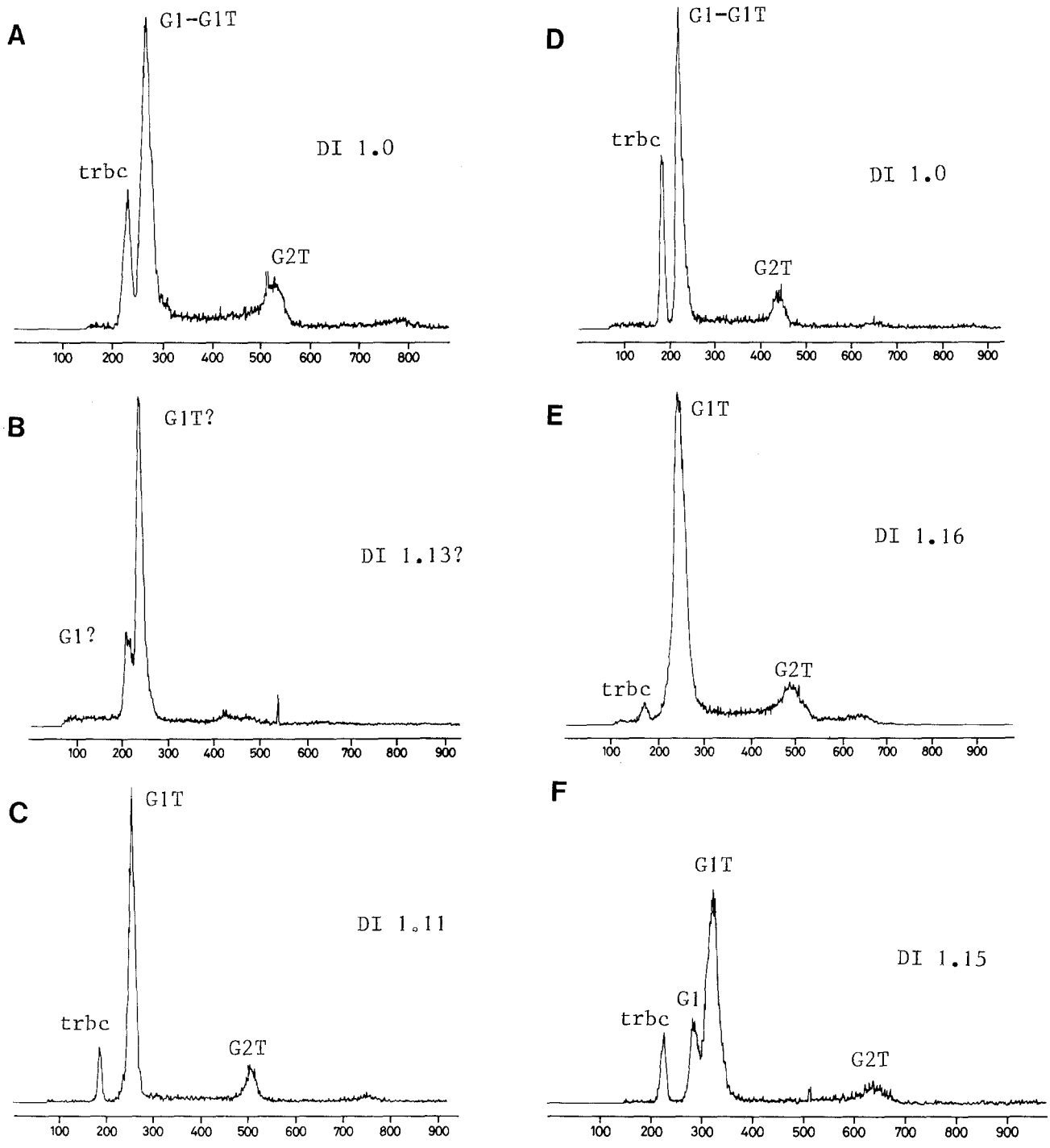



**Fig. 4.** DNA profiles from primary tumours (**A, C, E, G**) and metastases (**B, D, F, H**) or patients 1 through 4. **A, B:** patient 1; **C, D:** patient 2; **E, F:** patient 3 and **G, H:** patient 4. The number of fluorescent cells is plotted against channel numbers (relative DNA-contents). trbc: trout red blood cells; *G1* and *G1T*: normal and tumor cells, respectively, in the $G_{0\text{-}1}$ phase of the cell cycle; *G2T*: tumour cells in the G2M phase of the cell cycle; *DI*: DNA index

**Fig. 3. A, C,** primary tumours in cases 1 and 3, respectively. **B**, lymphogenous metastasis in case 1; **D**, haematogenous metastasis in case 3 (H&E ×140). Note that the blastematous component is more represented in the metastases than in the primary tumours

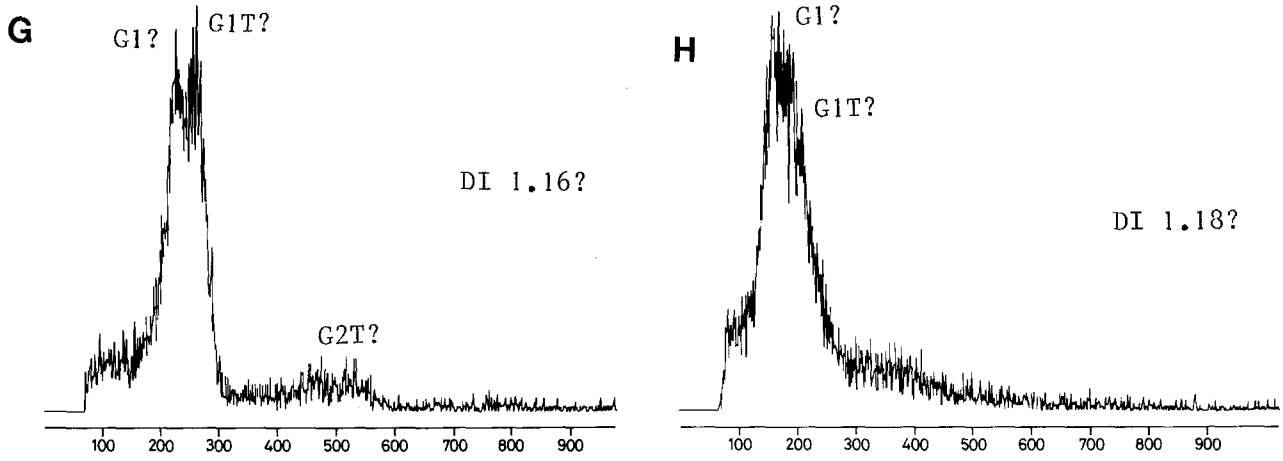



Fig. 4. G–H

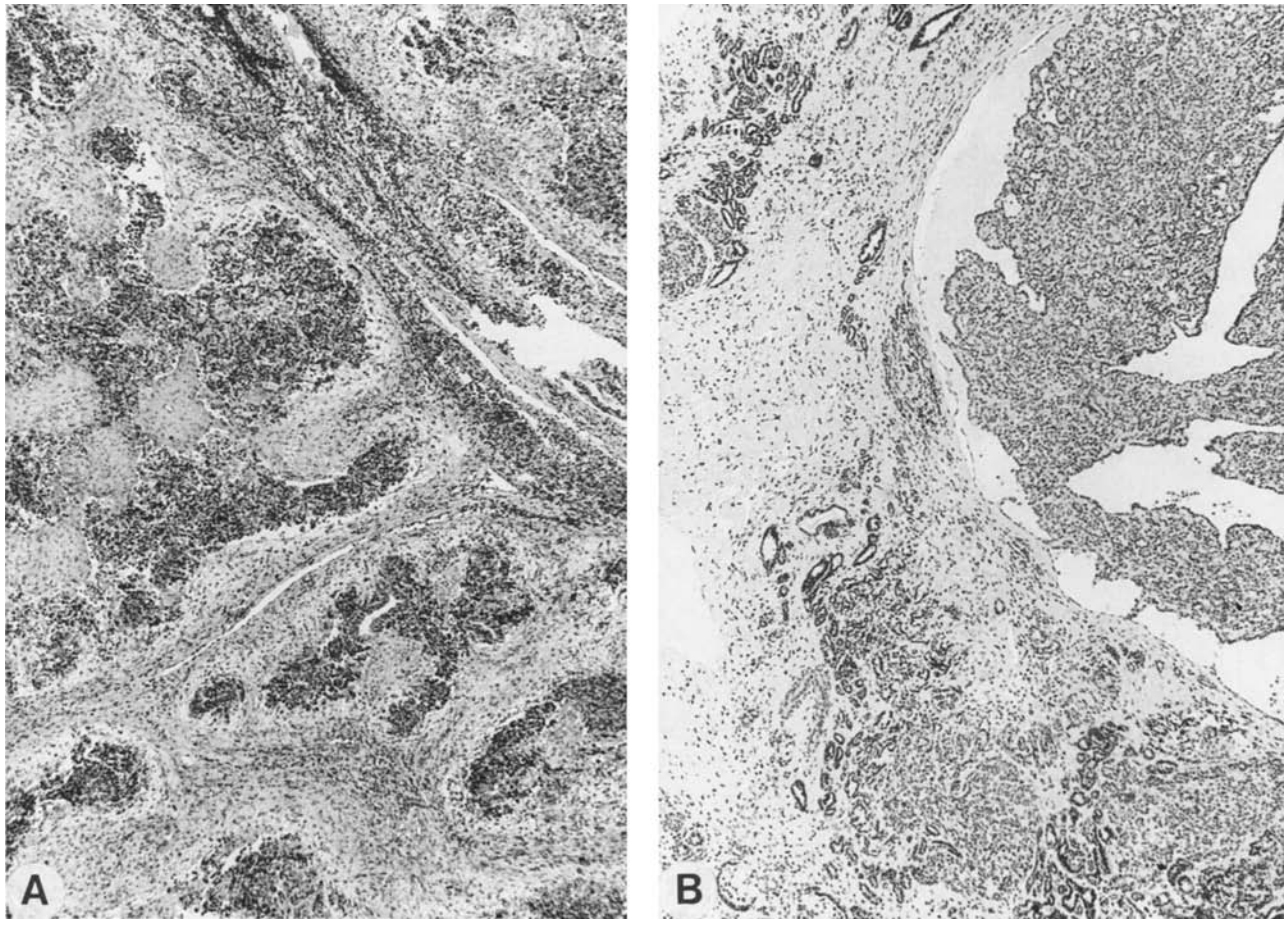



**Fig. 5.** **A** nephroblastoma and **B** nephroblastomatosis in the same child after 4 days of chemotherapy. The epithelial and stromal components of the nephroblastoma are still intact, while the blastematous component is necrotic. The nephroblastomatosis areas remained entirely unaffected (H&E ×56; inset ×160)

therefore reliable statistical evaluation is not feasible. However, the results in the various cases are consistent and it would be of interest to collect more data to test further the hypotheses bases on the present findings.

Thus, in all cases with viable metastases the blastema was the most important component and was relatively more represented in the metastases than in the primary tumour. A similar predominance of blastema in the metastases was also found

in a rabbit model of nephroblastoma (Hard and Fox 1983). Conversely, the stromal components were invariably and the epithelial components were almost always less represented in the metastases than in the primary tumour. In this small number of cases, the epithelial component tended to be more heavily represented in the lymphogenous metastases and the stromal component in the haematogenous metastases, a phenomenon also observed in stromal and epithelial components of synovial sarcoma (Evans 1983). Our findings might explain the earlier findings that a prominent epithelial component correlates with a better survival (Green and Jaffe 1978; Lawler et al. 1975; Lawler et al. 1977).

The DNA-ploidy of at least 2 of the 4 primary tumours was in the hyperdiploid range, similar to the findings of Tanaka et al. (Tanaka et al. 1983). Despite the histological observation of different tumour components, only one peak was found within the resolution limits of the present technique, this may indicate that morphologically different components have similar DI's, as was found in non-seminoma testis tumours (Oosterhuis et al. 1985). The finding that at least one, and presumably 2 metastases had similar DI's as the primary tumours, although the histology was different, also points in this direction. In patient 2, however the primary tumour was hyperdiploid, and the metastasis diploid, although the karyotype was very abnormal (Molenaar et al., submitted), indicating a pseudodiploid pattern. In patient 1 the primary tumour was diploid whereas the DNA profile of the metastasis suggests hyperdiploidy. These differences between the primary tumours and their metastases suggest selection of one metastasizing clone. The latter may have been present in the primary tumour but be too small to be detected in the DNA-profile. It is of interest that in case 2 the primary tumour, but not the metastasis, contained an anaplastic component which is likely to have an aneuploid DNA-profile. Finally, diploidy does not necessarily correlate with a better clinical behaviour (Barlogie et al. 1983; Friedlander et al. 1984) and may also be found in metastatic disease (Hedley et al. 1985). In this respect the findings of Look et al. (1984) in neuroblastomas who found significantly more diploidy in advanced than in early clinical stages are relevent, in their study hyperdiploid tumours appeared to respond better to chemotherapy than diploid tumours.

Morphological analysis of four of the current cases suggests that therapy exerts its influence primarily on the blastematous component of Wilms' tumour as was described by Becht et al. (1983). This observation parallels the findings in non-seminomatous testicular germ cell tumours and rhabdomyosarcomas in which the least differentiated components are affected by chemotherapy, leaving the components with a capacity to spontaneous differentiation relatively unaffected (Molenaar et al. 1984; Oosterhuis et al. 1983). The observation that benign or possibly premalignant nephroblastomatosis foci remained, unaffected by chemotherapy, in the current case 10, further substantiates the concept that differentiation is inversily proportional to the sensitivity to therapy. It seems to disagree with the recent report of Heideman et al. (1985) concerning the reduction of nephroblastomatosis following chemotherapy. However, the amount of chemotherapy given was much higher and, moreover, their findings were not histologically documented and the reduction of kidney size may in part be attributed to decrease of malignant foci.

The findings that on the one hand blastema is the most likely component to metastasize and on the other that blastema is the most sensitive to therapy seems contradictory. However, it should be kept in mind that the current cases were selected on the basis of metastatic disease and thus represent a biased group. A more biologically plausible explanation is that if one clone in the blastema is resistent to therapy, this clone will be most likely to metastasize. This explanation is supported by the findings in cases four and five. In the former case, a comparison with the primary tumour before and after treatment suggests only a minor reaction of the blastematous component to therapy. Correspondingly, the metastasis consists predominantly of blastema. In the latter case, however, the reaction to therapy of the blastematous component seems to be extensive and thus the metastasis is entirely necrotic.

Both findings, in respect to the metastatic behaviour and of the effect of therapy in the primary tumour, point to a central role for blastema. It therefore seems justified to focus future research into therapeutical regimens on the aim of total eradication of the blastematous component in Wilms' tumours, which may prevent both metastatic disease and local recurrence leading to death.

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