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Flow-Cytophotometric Studies in Renal Carcinoma

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Summary. In 36 patients with renal carcinoma ploidy and cell cycle analysis of the tumour tissues by flow cytophotometry were performed. Considering the tumour stages pT, pN, and M no relationship between stage and DNA distribution could be established. With reference to the histological grading, grade I tumours showed only euploid DNA distributions, whereas grade II and III carcinomas exhibited both euploid and aneuploid DNA patterns. Whether ploidy analysis is correlated with the prognosis of the tumour disease remains to be determined.

Key words: Renal carcinoma, Grading, Tumour stage, Flow-cytometry.

Introduction

During recent years the idea of grading renal carcinoma has gained considerable interest [6, 9, 13]. However, the large number of grading systems proposed in Scandinavia [1, 7, 20], England [21], USA [14, 17] and Germany [9] makes a reliable comparison and assessment of the value of each system extremly difficult. In addition the low reproducibility of histological grading [20] may be a considerable handicap for its general applicability.

Recently the ploidy and cell-cycle analysis measured by flow-cytometry (FCM) was shown to correlate with the histological grading according to Syrjänen and Hjelt [20] as well as the prognosis [2].

Since 1980 we have performed FCM-measurement of tissue specimens from renal carcinoma. In this paper we therefore compare these DNA histograms with both the histological grading according to Hermanek et al. [9] and with the tumour stage.

Materials and Methods

The patients (24 males and 12 females) with a median age of 63 years (from 45 to 74 years) were staged according to the criteria outlined by the UICC [24]. After irradiation with 20 Gy on four consecutive preoperative days, radical tumour nephrectomy was performed. From the tumour specimens two 2 x 2 x 2 cm tissue blocks without evidence of haemorrhage or necrotic appearance were obtained for FCM analysis. Cell suspensions from parts of the two blocks were pooled. Further fixation, preparation and FCM analysis were performed exactly as previously described [16]. In samples with euploid DNA-patterns the relative amount of S-phase cells was calculated according to Stöhr et al. [19]. For histological evaluation, at least four additional regions of the tumour were examined. Slides were prepared and stained in a routine fashion. The criteria of histological grading were different histological pattern and growth (cord-pattern, tubular, trabecular, papillar or alveolar pattern), the nuclear structure (anisonucleosis, prominent nuclei, mitotic figures) and the cell type (clear cells, eosinophilic granular cytoplasm, spindle cells, sarcomatoid [18, 20]). The grade of malignancy was determined according to Hermanek et al. [9].

Results

Fourteen of 36 analysed specimens (39%) showed aneuploid DNA-distributions. In 63% of these samples with euploid DNA-patterns the percentage of S-phase cells was approximately 5% (5.7% ∓ 1.2%), the remaining samples showing very much higher values (14.4% ∓ 15.9%). Figure 1 shows a DNA histogram of a grade II renal carcinoma exhibiting euploid cell distribution not discernible from normal non-malignant cells. In contrast, another tumour of the same grade shows aneuploid stem lines as examplified in Fig. 2. The typical histological findings in grade II–III carcinomas are documented in Figs. 3–5. The overall results of our investigation did not show any correlation between pathological primary tumour stage (pT), regional lymph node metastatic spread at operation (pN) or distant metastatic spread (M) and ploidy pattern of DNA-distribution (Tables 1, 2 and 3). In addition to the criteria of DNA ploidy, eval-

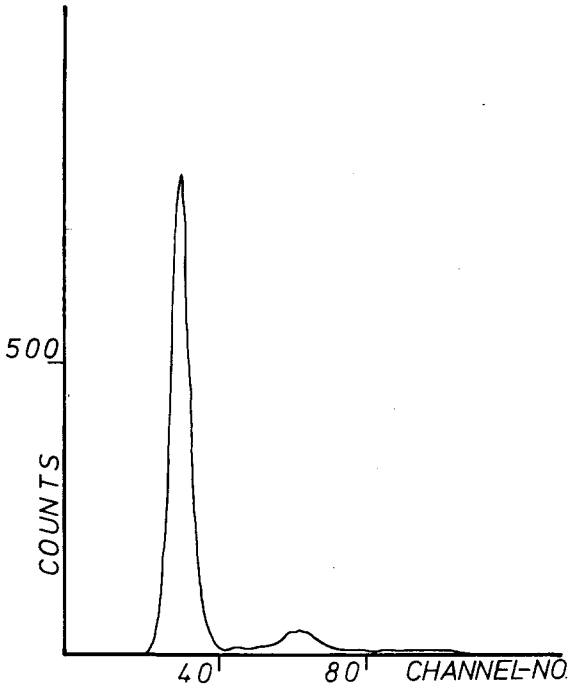

Fig. 1. DNA histogram with a euploid cell distribution in grade II pT2 pN0 M0 renal cell carcinoma

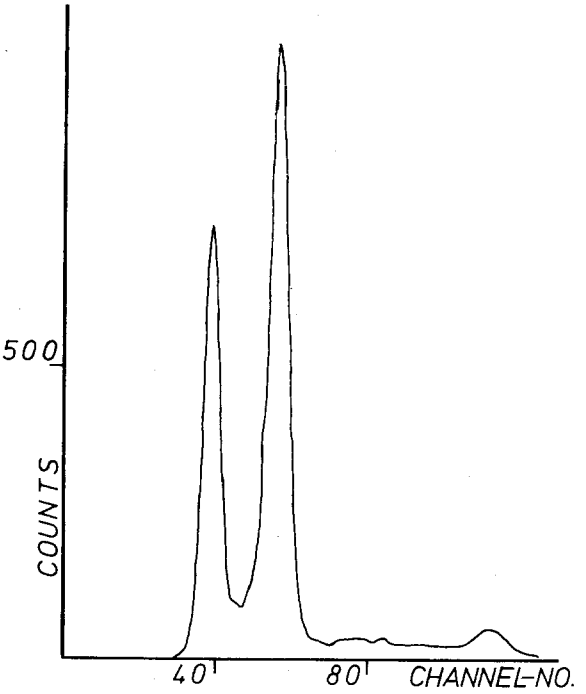

Fig. 2. DNA histogram with hyper-diploid tumour stem lines in grade II, pT2 pN0 M0 renal cell carcinoma

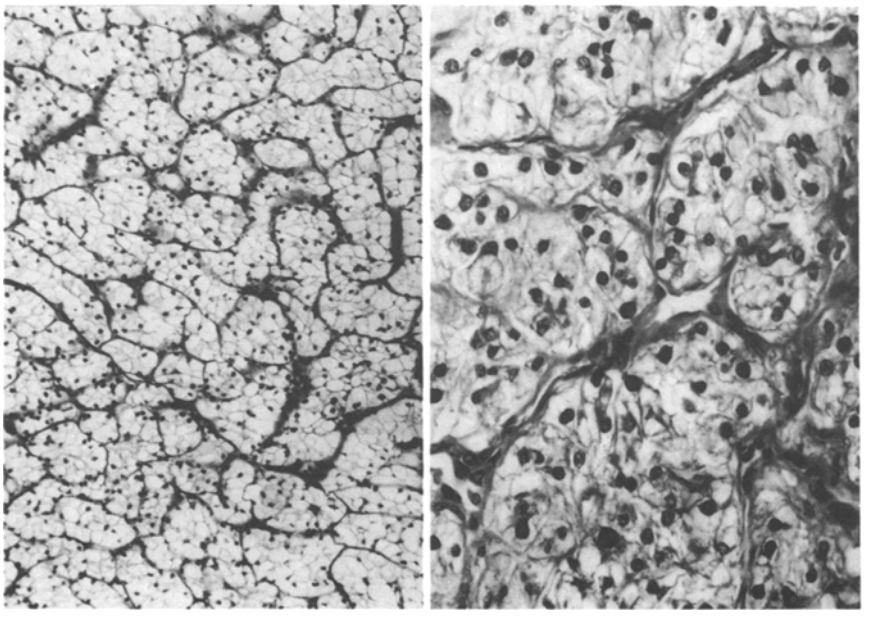

Fig. 3. Solid clear cell renal adenocarcinoma (grade I) (HE, x 61 and x 242)

uation of the S-phase of the cell cycle did not uncover any relationship to the tumour stages.

With reference to the tumour grade, G I tumours always exhibited euploidy whereas G II or G III tumours showed euploid as well as aneuploid DNA-patterns (Table 4). Also no relation could be found between tumour grade and the percentage of cells in S-phase.

Discussion

Ploidy analysis as well as determination of the proliferative state by FCM has been performed in a variety of solid non-urological tumours [3, 5, 12]. In urinary bladder cancer, DNA cell cycle analysis provided evidence that within the group of histological grade II tumours, euploidy is associated

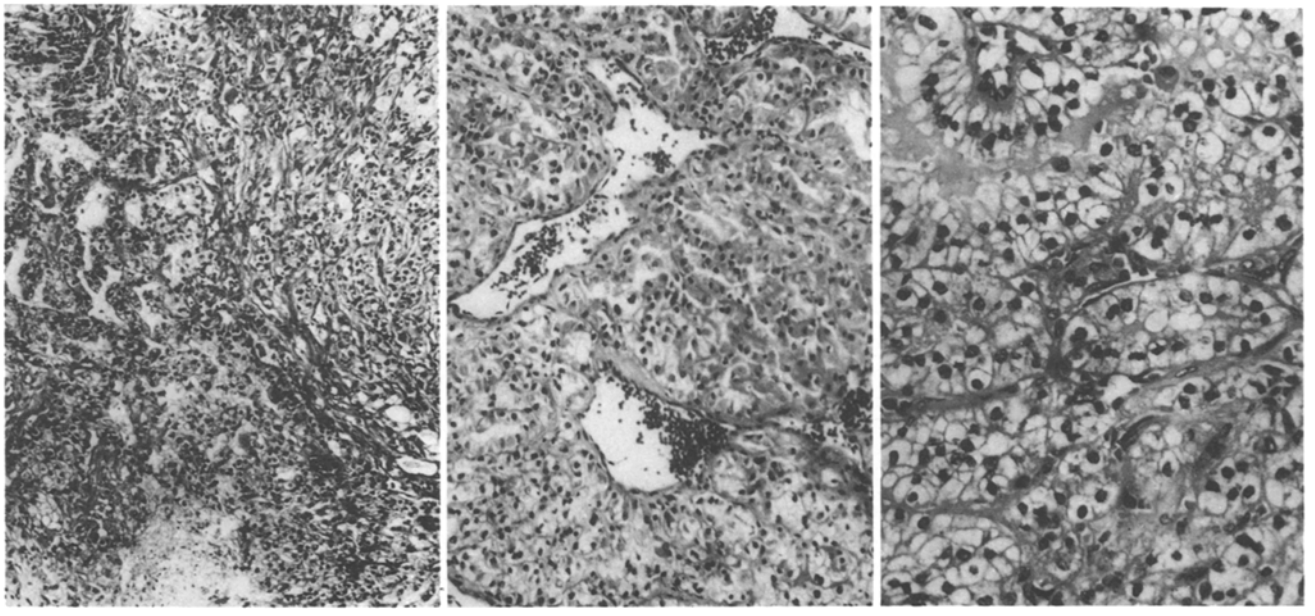

Fig. 4. Renal adenocarcinoma (grade II): clear cells (a), finegranular areas (b) as well as adenomatous and adenopapillary (c) differentiation (HE, x 61 and x 254)

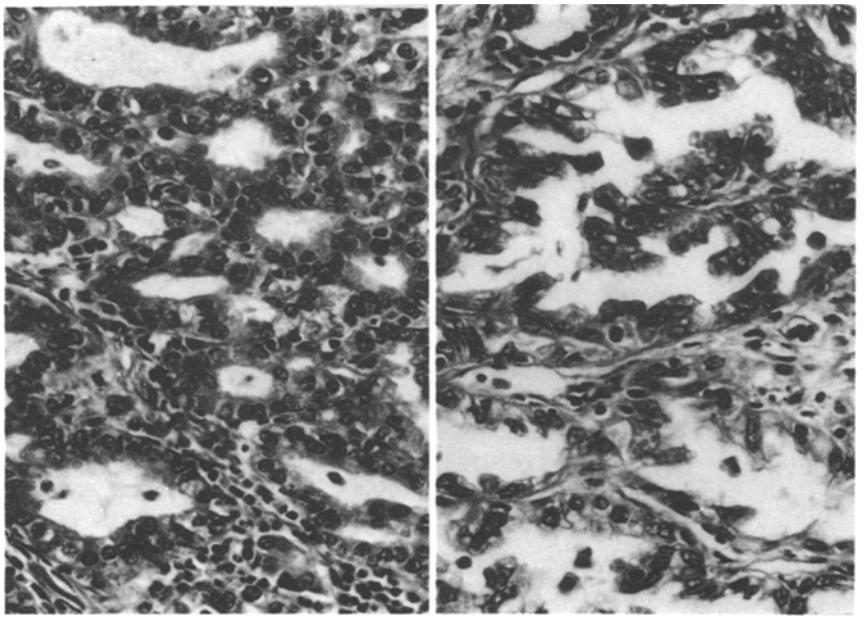

Fig. 5. Renal adenocarcinoma (grade III): exclusively tubular (adenomatous) (a) and adenopapillary (b) structures, nuclear polymorphy (HE, x 250)

Table 1. Relationship between the pT-stage (histopathological stage of the primary tumour) and the DNA ploidy

Ploidy	Stage (UICC 1979)			
	pT_1	pT_2	pT_3	pT_4
Euploidy	1	5	14	2
Aneuploidy	1	7	5	1

Table 2. Relationship between the pN-stage (histopathological stage of lymph node metastases) and the DNA ploidy

Ploidy	pN-stage (UICC 1979)	
	pN_0	pN_{1-3}
Euploidy	21	1
Aneuploidy	12	2

Table 3. Relationship between the M-stage (distant metastases) and the DNA ploidy

Ploidy	M-stage (UICC 1979)	
	M_0	M_1
Euploidy	16	6
Aneuplodiy	12	2

Table 4. Relationship between the grade of malignacy and the DNA ploidy

Ploidy	Grade (Hermanek et al. 1976)		
	I	II	III
Euploidy	6	14	2
Aneuploidy	0	11	3

with a more favourable prognosis whereas aneuploidy indicates a lower incidence of survival [8, 11, 22].

The results of our ploidy and cell cycle measurements do not show any relation to the primary tumour stage or to lymph node involvement or distant metastatic spread. In the literature FCM analysis or nuclear DNA content are only investigated in comparison to the grade of malignancy but not to the tumour stage [2, 10].

Grading of renal carcinoma implies several problems. Many grading systems have been proposed which are principally based on nuclear characteristics with or without considering the demarcation of the carcinoma from the surrounding tissue [20], different histological patterns [4, 9, 15, 18] or combinations of both [17, 20].

Although the specimens for histological grading were taken from different parts of the tumours, Syrjänen and Hjelt claim to be able to determine the predominating area of tumour differentiation [20]. Nevertheless, in their hands the reproducibility in grading renal carcinomas was only 50% [20]. Therefore the pluriformity of this tumour seems to be an unsolved problem with regard to its biological significance. At present no data are available for drawing meaningful conclusions about whether the survival rate depends either on the relative amount of each compartment of tissue differentiation or just on the most anaplastic grade independent of the respective tissue mass. For that reason serial giant sections with meticulous mapping of the different areas of tumour differentiation in relation to other relevant tumour parameters as well as to the prognosis is the only way of providing answers to these questions.

As long as no generally accepted and reproducible histological grading exists, FCM analysis of renal cell carcinoma will be problematical. Although in our study tumour cells from two different small areas were qualitatively analysed, under no circumstances can these DNA measurements be considered representative of the entire, sometimes very bulky, tumour. These problems must always be faced while interpreting FCM studies of renal tumours. With these limitations in mind, our results only show a relationship between euploidy and grade I tumours. The other grades of malignancy do not show any correlation with the pattern of DNA distribution. Also the percentage of S-phase cells indicating the proliferative activity of the tumour is not associated with the grade of malignancy. Our results contrast with those published by Baisch et al. [2], who reported a good correlation between tumour grade and FCM DNA ploidy. This discrepancy may partly be explained by a different grading system used and other techniques of DNA cell cycle analysis, particularly with regard to the computer programme employed for fitting of the histograms. Also Hofstädter and Ehlich [10] reported a correlation between the histological grading [9] and the DNA distribution measured by Feulgen cytophotometry. Obviously preoperative irradiation as performed in all of our cases has no effect on FCM determination [2]. Since in our study the follow-up is too short, prognostic conclusions cannot be drawn yet. Interestingly Baisch et al. [2] described a positive correlation between ploidy measured by FCM and prognosis of the tumour disease. The theoretical assumption based on the usefulness of this method in this regard would be that each small area of tumour tissue taken for FCM analysis would contain a representative number of cells displaying the typical pattern of DNA changes which represent the biological behaviour of the entire tumour. In this context it is interesting to note that in histologically rather uniform urothelial bladder cancer, ploidy studies proved to be more reliable in assessing the prognosis than conventional histological grading [8].

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