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Prognostic Influence of Ploidy Level and Histopathologic Differentiation in Cervical Carcinoma Stage Ib

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Abstract—*Flow-cytometric DNA analysis and extended histopathologic grading were performed in specimens from 126 patients with squamous cell carcinoma of the uterine cervix stage Ib. Archival material was used for the measurements and the ploidy level was analysed according to the method described by Hedley with some modifications. The histopathologic grading was based on eight well-defined parameters all scored 1–3. The results showed that the ploidy level held significant prognostic information about the 10 year survival according to a division of DNA indices above and below 1.5. Further prognostic information appeared from a combination of DNA index and histopathologic score value. The combination held its prognostic importance in subgroups of patients with different tumour sizes. It is concluded that flow-cytometric analysis and histopathologic grading can identify subsets of patients who need more aggressive treatment.*

INTRODUCTION

EARLY cervical carcinoma has a relatively favourable prognosis. The 5-year survival is in the order of 90% in most materials. Both surgery and radiotherapy can be applied in the treatment, alone or in combination. The cure rate appears to be largely independent of treatment type when proper selection of patients for surgery is applied. The prognosis is more related to the tumour size, patients with small tumours having the best chance of cure irrespective of treatment. The occurrence of lymph node metastases is also of prognostic importance, but can only be properly evaluated by operation. New parameters which can be used in the primary examination of the patients are needed to identify subgroups of patients with good or poor prognosis in order to offer the latter group a more aggressive treatment.

The DNA content of the tumour cells as analysed by flow cytometry appears to be a prognostic factor in a number of different malignant tumours [1, 2]. In previous studies we have shown that this also applies to cancer of the cervix [3]. The histopathologic differentiation also holds prognostic information [4] but ploidy level and histopathologic grade are independent prognostic parameters [5]. However, these investigations included patients with different stages all treated with radiotherapy. The present study was undertaken to explore the prognostic importance of ploidy level and histopathologic differentiation in early cervical carcinoma treated with surgery.

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MATERIALS AND METHODS

The patient material included 126 patients with squamous cell carcinoma of the cervix uteri. All patients were classified as stage Ib according to FIGO criteria. The tumour size in its largest dimension was estimated at the gynaecological examination under narcosis in 118 patients. Sixty-six patients had tumours <2 cm. The tumour size was 2–5 cm in 45 patients and exceeded 5 cm in seven patients. Subsequently, all patients underwent extended hysterectomy and careful lymph node dissection [6]. In the post-operative period an active regimen with early mobilization and respiratory exercise was applied.

Histopathologic grading

The specimens for histopathologic grading and flow-cytometric analysis were cut from the formalin-fixed uterus. A 5 μm section was cut for histopathologic grading followed by two 30 μm sections for flow-cytometric analysis and another 5 μm section

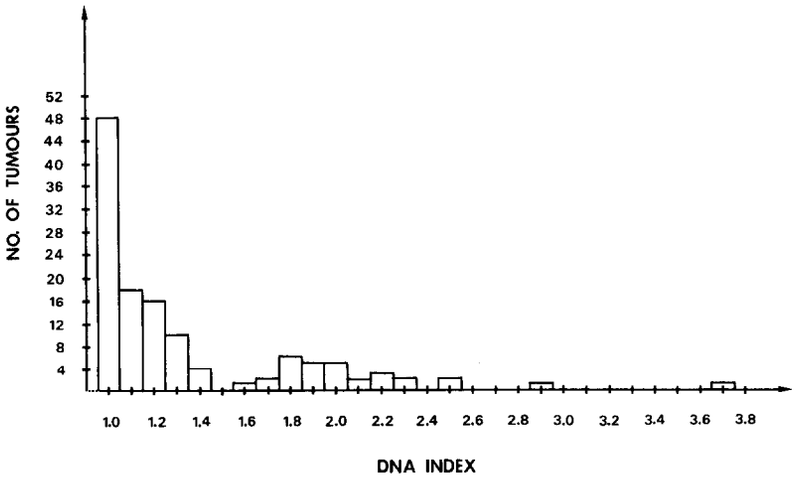

Fig. 1. Distribution of DNA indices in 126 patients with cervical carcinoma stage Ib.

for histopathology. This procedure ensured comparable specimens for grading and flow cytometry. The grading was performed on routine-prepared sections stained with haematoxylin–eosin.

The grading system used has been described in detail elsewhere [4]. In short, the system is based on eight different parameters (1: structure, 2: different cell types, 3: nuclear polymorphism, 4: mitosis, 5: mode of invasion, 6: stage of invasion, 7: vascular invasion, 8: cellular response). All parameters were scored semiquantitatively 1–3. The maximum score value (MGS index) was thus 24 and the minimal MGS index 8. According to our previous investigations, the tumours were divided into two groups with MGS index ≤ 14 and >14.

Flow cytometry

The specimens were prepared for flow-cytometric analysis according to the method first described by Hedley *et al.* [7] with some modifications. The sections were dewaxed, rehydrated and treated with 0.5% pepsin as described in the original method, but the staining was accomplished in a hypotonic solution containing Nonidet P-40 0.03%, RNase 0.001% and ethidium bromide 0.001% as described previously [8]. The staining time was 20–24 h. In our hands this procedure resulted in DNA histograms with a somewhat higher resolution than the original staining with DAPI. The flow cytometer was the microscope-based instrument described by Lindmo and Steen [9] built in our own laboratory [10]. The first peak in the DNA histogram was considered to represent normal cells and diploid tumour cells in the $G_{0/1}$ phase. The histopathologic examination ensured that lymphocytes and other stroma cells were represented in the sections adjacent to the specimens used for flow cytometry. The DNA index (DI) of a cell population was calculated from the ratio between the first peak and other peaks in the DNA histogram with the first peak normalized to 1.0. In most cases the coefficient of variation (cv) was 3–4% and a few cases with a cv $> 6\%$ were excluded.

Statistics

The survival analyses were performed by use of the BMDP program implemented on a Cyber 600 computer at the regional EDP centre at the Aarhus University. The survival curves were calculated as Kaplan–Meir plots and analysed for differences by use of the log-rank test.

RESULTS

The frequency distribution of DNA indices is shown in Fig 1. The tumours clearly fall into two groups with a DI above and below 1.5. The group with a low DI appears well-defined, whereas the

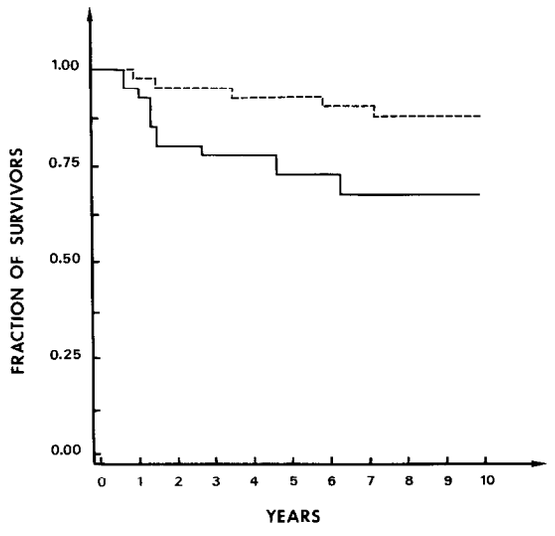

Fig. 2. Survival according to ploidy level. --- DI $\leq$ 1.5. —— DI $>$ 1.5. P = 0.004.

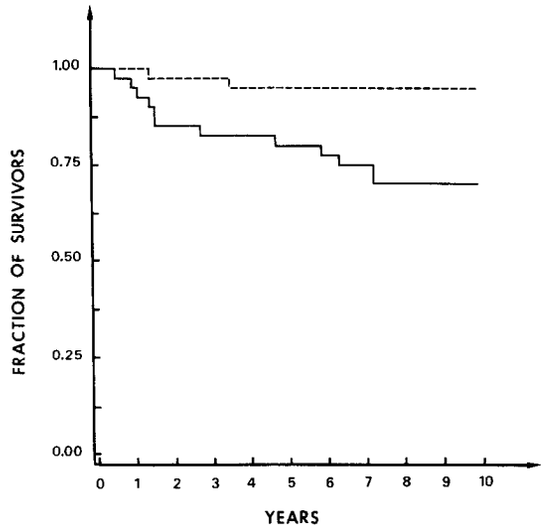

Fig. 3. Survival according to ploidy level and histopathologic differentiation. --- DI ≤ 1.5 and MGS index ≤ 14. —— DI > 1.5 or MGS > 14. P = 0.002. The two groups constitute 67 and 59 patients, respectively.

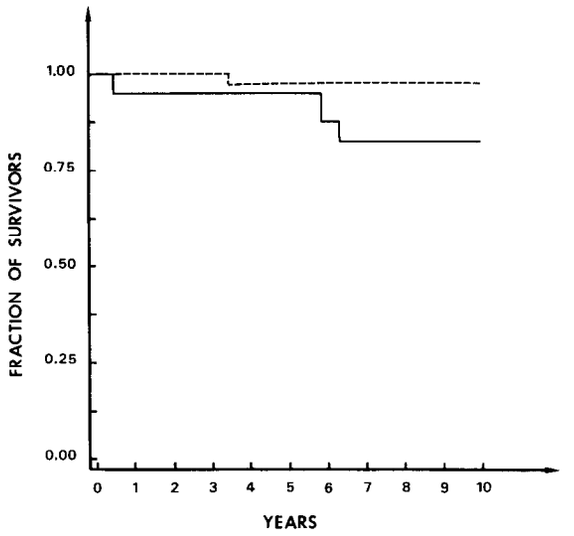

Fig. 4. Survival according to ploidy level and histopathologic differentiation in 66 patients with tumours < 2 cm. --- DI ≤ 1.5 and MGS index ≤ 14. —— DI > 1.5 or MGS index > 14. p = 0.07.

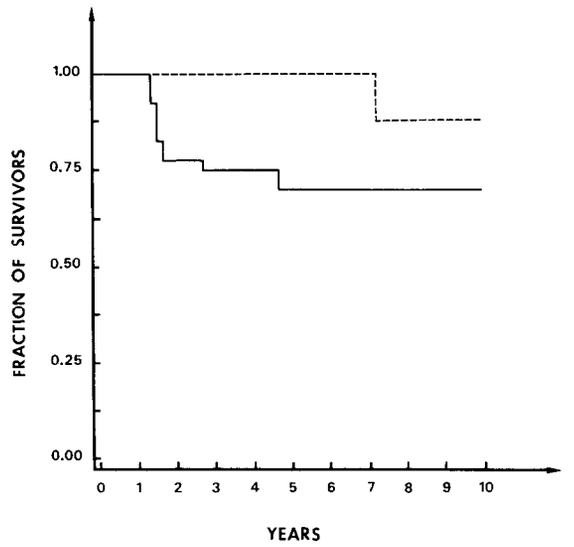

Fig. 5. Survival according to ploidy level and histopathologic grading in 45 patients with tumours 2–5 cm. --- DI ≤ 1.5 and MGS index <14. —— DI > 1.5 or MGS index > 14. p = 0.06.

group with a high DI is rather heterogeneous and scatters over a wide range.

Figure 2 shows the survival according to ploidy level. The patients were divided into two groups according to the distribution of tumour DNA indices suggested by Fig. 1. The significant difference between the Kaplan–Meir plots cannot be explained by difference of age in the two groups. The median age was 47 and 43, respectively.

Further prognostic information appeared by combining the ploidy level and MGS index as shown in Fig. 3. In the group of patients (67) with a DI ≤ 1.5 and a MGS ≤ 14, only three patients died compared with 14 in the group (59 patients) with a DI > 1.5 or a MGI > 14. Consequently, the cumulative survival at 10 years was 95% and 69%, respectively.

The prognostic difference might be attributed to a different distribution of tumours of different sizes in the two groups. Further analysis of different subgroups, however, discounted this possibility. Figure 4 shows the survival of patients with tumours < 2 cm.The division according to DI and MGS used above still holds considerable prognostic information although marginally significant. Analysis of patients with tumour size 2–5 cm shown in Fig. 5 confirmed the results. The worse prognosis was found in patients with a high DI or a high MGS index.

DISCUSSION

The clinical application of flow-cytometric DNA analysis is still a matter of debate. The modern technique with internal standardization has provided reliable measurements [11] and the method described by Hedley *et al.* has opened the possibility of utilizing archival material. However, the literature on the prognostic implication of ploidy level describes diverging and to some extent contradictory results. The division into diploid vs. aneuploid tumours used in many studies is probably not the best one. Thus, considerable deviations may still be concealed in the genetic material of tumours assigned diploid even by high resolution flow cytometric analysis. Consequently, diploid tumours cannot in advance be considered less malignant than aneuploid tumours. Other authors have divided tumours into euploid and aneuploid categories and claimed a better prognosis for the euploid group [12, 13]. This division may also be criticized as e.g. tumours with a tetraploid DNA content are not

necessarily tetraploid from a genetic point of view. The frequency distribution of DNA indices is bimodal in most tumour types with a group of tumours in the diploid and near-diploid region and another group in the hypertriploid area. This distribution may be accounted for by assuming that tumour development is conditional upon a range of different mechanisms. Tumours with a near-diploid DNA content may arise by a gain of chromosomes, e.g. non-disjunction, whereas the hypertriploid DNA content may be explained by tetraploidization and subsequent loss of chromosomes. This hypothesis could explain the relatively small number of tumours with triploid DNA content found in most studies, and it might also support the assumption that the two groups have different prognosis. The non-traditional division of DNA indices used in the present study speaks in favour of this theory and is in agreement with our previous results on cervical carcinoma. The division has also been used in a few other studies [14, 15].

Flow-cytometric DNA analysis is probably useful as a prognostic parameter, but it should be combined with other independent parameters to allow a more accurate prognostication. The extended grading system appears to increase the prognostic information and its independency of ploidy level has been proved in earlier studies [5]. Furthermore, the combination of DNA index and histopathologic score value holds its prognostic value in subgroups of patients with different tumour sizes, underlining the fact that these parameters do not merely reflect the extension of the tumour.

Ideally, any patient material used for assessment of new prognostic parameters ought to be strictly homogeneous with regard to all other prognostic parameters including treatment, but in practice, uncertainty about some prognostic factors prevails in practically all cancer patients. The present study included patients with the same FIGO stage which is a major prognostic factor in cervical carcinoma. It also comprised only one histopathological type and all patients underwent the same treatment.

The present work confirms our previous results in patients treated with radiotherapy. It shows that even if patients with early cervical carcinoma as a group can be considered to have a good prognosis when treated by surgery a subgroup can be identified that has a rather poor survival (tumour size > 2 cm, DI > 1.5 or MGI > 14). It seems reasonable to suggest that these patients should be offered a more aggressive treatment.

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