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# Ploidy Level, Histopathological Differentiation and Response to Chemotherapy in Serous Ovarian Cancer

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**Abstract**—*The ploidy level was measured in tumour specimens from 64 patients with advanced epithelial ovarian cancer (FIGO stages III and IV) by flow cytometric analysis. The same tumours were histopathologically graded based on a score system considering eight histopathological parameters. The results showed that both the histopathological grading index (HGI) and the DNA index were important to the frequency of complete pathological remission as assessed by second look operation. A combination of the two parameters allowed a division of the patients into three groups with different frequencies of complete pathological remission and survival. It is concluded that flow cytometric analysis and extended histopathological grading may be important adjuncts in the evaluation of malignant epithelial ovarian tumours.*

## INTRODUCTION

CHEMOTHERAPY in different combinations is now the therapy of choice in advanced ovarian cancer. Different regimens, especially those containing *cis*-platinum, yield high response rates [1] and improve survival in at least subgroups of patients. The effect of chemotherapy is, however, difficult to evaluate by clinical examination. Complete pathological remission (CPR) assessed by second look operation and long-term survival are the only reliable parameters of effect. Second look operation is a strain to the patients and there is an obvious need for new parameters which alone or combined can be used to predict the effect of chemotherapy.

Conventional histopathological grading of the tumours according to the WHO or Broder systems apparently provides some prognostic guidance although neither of the systems is ideal and improvements by the introduction of more detailed histopathological criteria can probably be obtained.

The distribution of DNA content in the tumour cell population as measured by flow cytometric analysis is likely to be of considerable importance [2, 3], but its correlation with response to cytostatic treatment has not been elucidated in any detail.

The purpose of the present study was to investigate the possible relation of a new histopathological grading system and ploidy level with the effect of chemotherapy as assessed by the frequency of CPR at second look operation and survival.

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## MATERIALS AND METHODS

The study included archival biopsy specimens from 64 patients with serous ovarian cancer FIGO stages III [4] and IV [5] registered in The Danish Ovarian Cancer Study. All patients underwent laparotomy with hysterectomy, bilateral salpingo-oophorectomy and omentectomy when possible. In all cases efforts were made to perform debulking surgery. At the operation the patients were divided into two groups with a residual tumour $\leq 1$ cm and $>1$ cm, respectively.

Following informed consent, the patients entered a randomized trial comparing 12 courses of cyclophosphamide doxorubicin and *cis*-platinum (CAP) or the same regimen without doxorubicin (CP). The two regimens did not result in difference of survival and the clinical trial is thoroughly described elsewhere [6]. At the end of treatment (1 year) all patients underwent second look operation to evaluate the response and remove any residual tumour when indicated. The operation included careful inspection of the abdominal cavity and biopsy specimens from all suspect areas. Furthermore, a peritoneal lavage was performed. Based on the results of the histopathological examination, the patients were divided into two groups with or without CPR, the latter group also included patients with malignant cells in the peritoneal lavage.

### *Histopathological grading*

The new system was described in detail previously [4]. It considers both the architectural features of the tumour, the cytological characteristics and the tumour-host relationships. The system is

based on the evaluation of the following eight parameters: structure, nuclear polymorphism, nucleolar features, nucleolar/cytoplasmic ratio, frequency of mitosis, mode of invasion, capsular penetration and vascular invasion. Each parameter was numerically scored from 1 to 3. The total score value thus ranged from 8 to 24 and the mean score (Histological Grading Index = HGI) from 1 to 3.

In the original work [4] a division of the tumours into two groups with a HGI ≤2 and >2 was shown to hold considerable prognostic information. The same division was therefore applied in the present work.

*Flow cytometry*

The method described by Hedly *et al.* [5] was used with a few modifications. Two 30 μm sections were cut from the paraffin-embedded blocks. A section for histopathological grading was cut on both sides of the 30 μm sections. This procedure ensured that sections for flow cytometry contained both normal and tumour cells and further allowed comparison of the histopathological grading and the flow cytometric analysis in the same area of the tumour. The specimens were dewaxed as described by Hedly *et al.* The staining was accomplished in a hypotonic solution containing Nonidet P-40 0.003% RNase 0.001% and ethidium bromide 0.001% for 20–24 h. The staining time was rather long because experiments indicated that a higher fluorescence would be obtained by overnight staining. Measurements were performed by use of the flow cytometer described by Lindmo and Steen [7] with minor modifications [8]. The first peak in the DNA histogram was considered to represent normal cells and diploid tumour cells. A DNA index (DI) was calculated by comparing different peaks in the histograms with the diploid peak normalized to 1.0. The coefficient of variation (cv) of the $G_0/G_1$ peak was ≤5%.

## RESULTS

The histopathological grading (HGI) is compared with the ploidy level in Fig. 1. The DNA histograms were divided into two groups with a DI ≤1.5 and a DI >1.5. Although the group with a DI >1.5 did not contain tumours with HGI values less than 1.7, a considerable overlap is obvious and the two distributions do not point to a correlation between histopathological differentiation and ploidy level.

Table 1 shows the frequency of CPR according to the tumour DNA index. The figures clearly indicate the predictive value of the ploidy level. The group with a DNA index ≤1.5 had a frequency of CPR of 55.5% v 30% in the group with a DNA index >1.5. The difference is statistically significant ($P < 0.05$).

The prognostic importance of the DNA index was demonstrated by the survival plots (Fig. 2), which were significantly different. This difference could not be ascribed to an uneven distribution of patients with residual tumour>1 cm v <1 cm. Nor can the different survival rates be explained by treatment, age or other prognostic parameters. A minor subgroup (nine patients) had tumours with two or more aneuploid cell populations. This group has a very poor prognosis as eight of these patients died within the period of observation.

The combination of DI and HGI appears from Table 2. According to a division of the tumours into

*Table* 1. *Frequency of CPR at second look operation according to DNA index*

| | DI ≤1.5 | DI >1.5 |
|---|---|---|
| CPR | 15 | 11 |
| Residual tumour | 12 | 26 |

$0.025 < P < 0.05$.

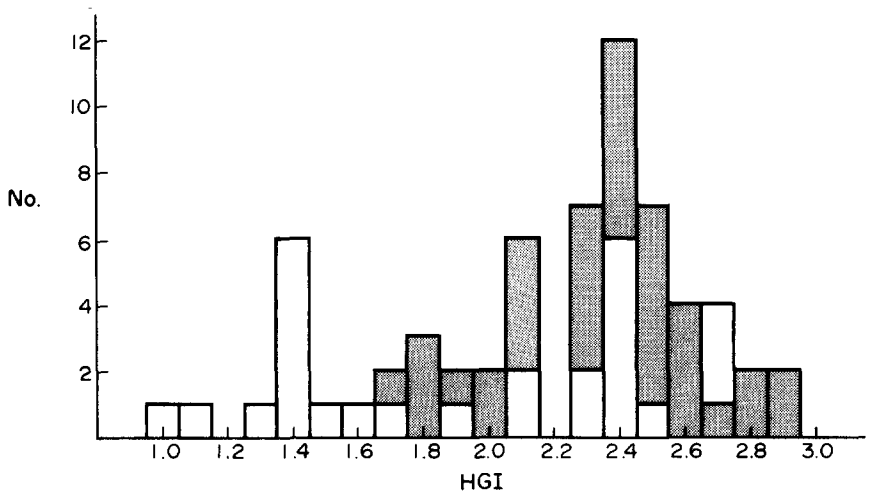


*Fig.* 1. *Distribution of HGI according to a division of DI into two groups with DI* ≤1.5 *and DI* >1.5, *respectively. Open: DI* ≤1.5. *Shaded: DI* >1.5.

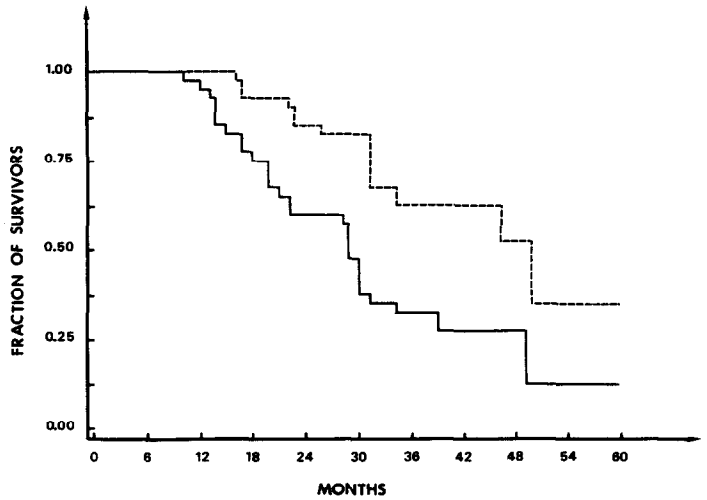


*Fig. 2. Survival of 64 patients according to DI. — — — DI ≤1.5. ——— DI >1.5. The difference is statistically significant (P = 0.006).*

*Table 2. Frequency of CPR according to HGI and DI*

| | DI ≤1.5 and HGI ≤2 | DI >1.5 or HGI >2 | DI >1.5 and HGI >2 |
|---|---|---|---|
| CPR | 8 | 11 | 7 |
| Residual tumour | 5 | 10 | 23 |

$0.025 < P < 0.05$.

two groups with a HGI ≤2 and >2 a significantly different frequency of CPR was found (results not shown), but a further subdivision based on a combination of the two parameters allowed a division into three groups with (1) a DI ≤1.5 and a HGI ≤2, (2) a DI >1.5 or a HGI >2 and (3) a DI >1.5 and a HGI >2. The frequency of CPR was significantly different ($P < 0.05$).

The survival of the three groups was also different (Fig. 3) despite the rather small numbers in each group. The Kaplan–Meier plots indicate the difference of death rates to be more pronounced for the first 3 years of observation.

## DISCUSSION

The treatment of advanced ovarian cancer faces several problems. Combination chemotherapy is rather toxic and its effect is only temporary in most patients. It seems reasonable to state that such hazardous treatment should only be offered to patients with a probable chance of cure. A major obstacle to a more individualized treatment is the lack of reliable predictors of effect.

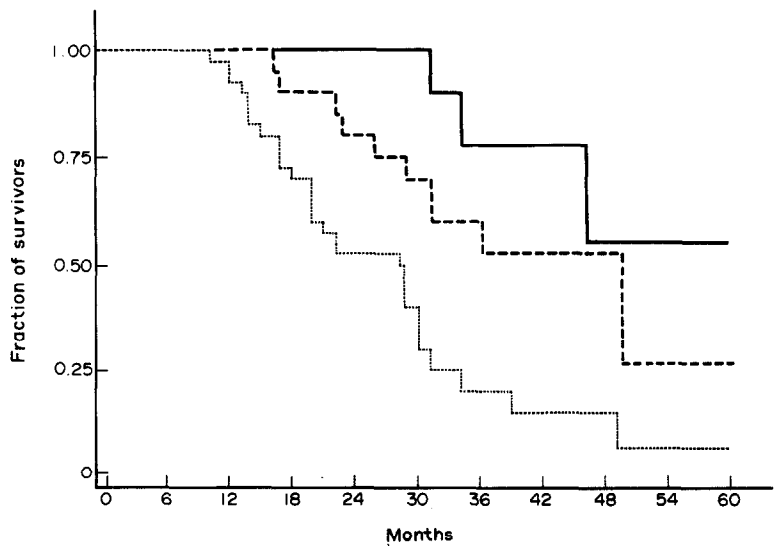


*Fig. 3. Kaplan–Meier plots showing survival of the patients divided into three groups. ——— DI ≤ .5 and HGI ≤ 2. — — — DI ≤ 1.5 or HGI > 2 . . . . DI > 1.5 and HGI > 2. The difference is statistically significant (P = 0.0002).*

The histopathological differentiation has been reported to be correlated with the prognosis in some studies [9, 10] although conflicting results have been published. The extended grading used in the present investigation appears to contain considerable information concerning both the response to chemotherapy and the final outcome. It is probably not surprising that the semiquantitative evaluation of eight histopathological parameters presents a more informative biological characterization than e.g. the WHO system. A similar system has proved its prognostic value in cervical carcinoma [11] and is now assessed by an international panel of pathologists.

A number of recent papers [12, 13] have described the prognostic value of flow cytometric DNA measurements in ovarian cancer. However, many studies are rather heterogeneous with respect to patient material and treatment, and it is not known whether the better prognosis of diploid tumours found in some investigations is explained by a better response to chemotherapy. All patients in our material underwent second look laparotomy and it may be objected that these patients represent a subgroup of advanced ovarian cancer with a favourable prognosis. The results may therefore not apply in general. On the other hand, the predictive value of the ploidy level and the HGI held true irrespective of tumour residue (<1 cm v >1 cm) at the primary operation. It should also be taken into account that only one specimen from each tumour was analysed and ovarian tumours are often heterogeneous at least from a classical histopathological point of view, but the ploidy level appears to be a stable marker [14] both in the primary tumour and metastases.

The prognostic importance in ovarian cancer of the DNA index according to a division above and below 1.5 has not been described previously. Most authors have applied a division into diploid and aneuploid tumour, but the results presented here are in agreement with our findings in cervix cancer [15, 16] and a discussion of the problem has been given elsewhere [17].

In correspondence with other studies a tendency for poorly differentiated tumours to have the highest DNA index was observed. There was, however, no clear correlation and the two parameters are likely to reflect different biological characteristics. This is in accordance with the fact that additional predictive and prognostic information was obtained by a combination of the parameters.

The present treatment of advanced ovarian cancer is by no means ideal. One way to obtain a more rational basis for treatment policy may be a better biological characterization of the tumours. A combination of prognostic parameters (prognostic index) may allow an individual treatment that of course should be investigated in prospective trials. Extended histopathological grading and flow cytometric DNA analysis seems to be a step in that direction.

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