Eur J Cancer, Vol. 29A, No. 3, pp. 337–340, 1993.
Printed in Great Britain

0964–1947/93 $6.00 + 0.00

Prediction of Lymph Node Metastases in Patients with Early Squamous Cell Carcinoma of the Cervix Uteri by Histopathological Grading and Flow Cytometry

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The present study comprises a retrospective investigation of 126 patients with stage IB squamous cell carcinoma of the cervix, and a similar prospective investigation of 53 patients with stage IB and 6 patients with stage IIA disease. Tumour biopsies from these patients were analysed by means of flow cytometry and a semiquantitative histological grading system. The study showed that a combination of a low tumour cell DNA index and a low score value of the grading system indicated a very low risk of regional lymph node metastases (0% lymph node metastases in patients with low scores vs. 24–46% metastases in patients with high scores, $P < 0.001$). In order to study the reproducibility of the histological grading 20 randomly selected cases were studied blindly by three of the participating pathologists and after discussion of the grading criteria. A kappa coefficient of 722 demonstrated a substantial agreement between the observers. These results suggest that by combining flow cytometry with semiquantitative histological grading, a subgroup of patients with early squamous cell cancer of the cervix uteri may be selected that could be sufficiently treated with simple hysterectomy instead of radical hysterectomy including lymphadenectomy, which, in many oncology centres, is the standard treatment of this patient category.
Eur J Cancer, Vol. 29A, No. 3, pp. 337–340, 1993.

INTRODUCTION

RADICAL HYSTERECTOMY is the standard treatment of patients with early cervical cancer, FIGO stage IB-IIA, at least in premenopausal women. A number of studies have shown that only 15–25% of the patients with early cancer of the cervix have lymph node metastases, and the majority of these women are therefore overtreated, often with considerable discomfort.

A less extensive surgical procedure without lymphadenectomy would eliminate most of the postoperative complications and would be advantageous for quite a number of women provided that patients with low risk of metastases could be preoperatively identified.

Previous studies have shown that the survival of patients with squamous carcinoma of the cervix is positively correlated with both the MGS histopathological grading system invented by Stendahl, (malignancy grading system) [1, 2] and the ploidy of the tumour cells [3, 4]. In a previous study [5] on patients with early cervical cancer it was possible to identify a group with no or very little risk of regional lymph node metastases by a combination of MGS grading and flow cytometric DNA analysis on preoperative biopsies of the tumour. In routine practice, however, the determination of the MGS score which is based on an evaluation of eight different histomorphologic parameters, will be considered a tedious task by most pathologists, and often it is impossible to perform a safe evaluation of all eight parameters because of too small size or inferior quality of the biopsy specimens. In previous studies a reduced version of the MGS, the partial index (PI) consisting of only four of the MGS parameters proved to be almost as strong in prognostic prediction as the MGS score [2, 6]. Therefore, the purpose of the present work was to investigate if a combination of the PI, instead of the MGS score value, and the tumour cell ploidy determined by flow cytometry in preoperative biopsies of the cervix could also be used to predict the presence of regional lymph node metastases in patients with early cervical cancer.

Like all other grading systems based on micromorphology, the MGS system is hampered by subjectivity in the evaluation. Although the MGS interobserver variation, according to previous investigations [7], seems to be reasonably low, the present work also intended to study the interobserver variation in two different series of tumour biopsies before and after a discussion of the grading criteria at the microscope by three of the participating pathologists.

MATERIALS AND METHODS

The study comprises a retrospective analysis of specimens from the cervix uteri of 126 consecutive patients with cervical squamous carcinoma, FIGO (1978) stage IB and preoperative biopsies from a prospective series of 59 consecutive patients with stage IB (53 cases) and IIA (6 cases) squamous cell carcinoma and in whom the bioptic material was quantitatively and technically sufficient to allow both a flow cytometric determination of the DNA index and a reliable histopathological grading. The total number of patients in the study was 220, but in

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Revised 1 Apr. 1992; accepted 6 Apr. 1992.

Table 1. Partial index

Parameter	Points 1	2	3
Mitosis	Single 0–1/HPF	Moderate 0–5/HPF	Numerous 0–>5/HPF
Mode of invasion	Well-defined borderline	Cords, less-marked borderline	Groups of cells or diffuse growth
Vascular invasion	None	Possible	Well established within the lumen of lymph or blood vessels
Cellular response (plasmo-lymphocytic)	Marked (continuous rim)	Moderate (several large patches)	Slight or none (few small patches or no cells)

35 cases (16%) the biopsy specimens were either too small or of poor technical quality, making grading and/or flow cytometry impossible.

All patients underwent radical hysterectomy (Meigs modification of Wertheims radical hysterectomy with dissection of the lymph nodes along the common and external iliac vessels, the presacral and the nodes in the obturator fossa). For histopathological grading all specimens were embedded in paraffin, prepared according to routine histological techniques and stained with haematoxylin–eosin.

In the retrospective study 30-μm-thick sections were cut from the paraffin-embedded tissue, together with the section for histopathological grading, and prepared for flow cytometric DNA analysis according to a previously described modification [8] of the method invented by Hedley [9].

In the prospective study two biopsy specimens, immediately adjacent to each other, were taken, one for grading and one for freezing (−80°) and future analysis by flow cytometry as described in detail elsewhere [10]. The suspension of single nuclei were stained with ethidium bromide and analysed in a mercury lamp-based flow cytometer. The coefficient of variation (CV) in the fresh tissue was 2% and in the paraffin-embedded material was 3.5%. Samples with a CV of more than 5% were excluded. The histopathological grading was based on a semiquantitative score system, MGS, an eight parameter system (P1–P8), which has been described in detail previously [1, 2].

The PI is a reduced version of the MGS system and defined as the sum of the parameters P4 = frequency of mitosis, P5 = mode of invasion, P8 = lymphoplasmocytic infiltration and two times the score value of P7 = vascular invasion. Each parameter is evaluated in terms of a 1–3 score (Table 1). The total score variation of PI is 5–15. A very low discriminating factor (PI ≤ 7) was chosen in order to ensure that only patients with histologically very well differentiated tumours were included, and according to previous studies [6] patients with a low PI have by far the best prognosis.

The single cell DNA content of the tumour cells was determined by means of flow cytometry and the tumours were classified according to a DNA index ≤ 1.5 and > 1.5 as previously described [3, 4, 10]. The DNA analysis and the histopathological grading were performed without knowledge of the patients lymph node status, which was examined by the pathology departments of the five participating Danish oncology centres.

The difference between the groups of patients with and without lymph node metastases was analysed with the χ^2 test. [In general, 5–8 lymph nodes were examined by simple microscopy, range 2–20. Large lymph nodes (>5 mm) were serially analysed].

The interobserver variation in the PI score was evaluated by kappa statistics in which the observed agreement is adjusted for chance agreement [11, 12]. $\kappa = 1$ means full agreement, $\kappa = 0$ is found when agreement can solely be explained by chance and $\kappa < 0$ is found when the observed agreement is less than expected by chance, i.e. the observers disagree.

The reproducibility in the PI grading was investigated in 20 randomly selected cases in which the PI was determined blindly by three of the participating centre pathologists (PB, KN, BH). The grading was performed without instructions and with a knowledge of the MGS system limited to what can be read in the literature. Then a number of cases were discussed in common under the microscope to sharpen the grading criteria of the PI parameters. A new series of 20 cases were then evaluated blindly by each of the three pathologists. The patients were divided in two classes—1:PI ≤ 7 and 2:PI > 7.

RESULTS

Table 2 shows the results of the retrospective study. None of the patients with a DNA index ≤ 1.5 and a PI score ≤ 7 had metastases in the regional lymph nodes. In contrast, 24% of the patients with a DNA index (DI) > 1.5 and/or a PI score of 8 or more had lymph node metastases. The prospective study confirmed the results of the retrospective series (Table 3). The difference in metastatic frequency between the two groups was highly significant. There was no difference between patients with stage IB and stage IIA disease. Taken alone the PI score appears to have stronger predictive value than the DNA index.

Table 4 shows the agreement between the three investigators prior to and after the mutual discussion of the grading criteria. It appears that in the first series of cases the agreement between the three observers was moderate ($\kappa = 0.594$), while after discussion the agreement in the second series increased considerably (overall agreement 0.933, $\kappa = 0.722$).

DISCUSSION

Radical hysterectomy with the obligatory extirpation of the pelvic lymph nodes has serious complications [13]. Bearing in

Table 2. Retrospective study of the correlation of DI and histopathologic PI score to frequency of lymph node metastases in 126 patients with stage IB cervical carcinoma

	Total number of patients	With lymph node metastases	Without lymph node metastases
PI ≤ 7 and DI ≤ 1.5	47	0 (0%)	47 (100%)
PI ≤ 7 and DI > 1.5	5	0	5
PI > 7 and DI ≤ 1.5	50	10	40
PI > 7 and DI > 1.5	24	9	15
PI > 7 and/or DI > 1.5	79	19 (24%)	60 (76%)

$\chi^2 = 13, P < 0.001$ (PI ≤ 7 and DI ≤ 1.5–PI > 7 and/or DI > 1.5).
DI = DNA index. PI = Partial index.

Table 3. Prospective study of the correlation of DI and PI score to frequency of lymph node metastases in 59 patients with stage IB and IIA cervical carcinoma

	Total number of patients	With lymph node metastases	Without lymph node metastases
PI ≤ 7 and DI ≤ 1.5	18	0 (0%)	18 (100%)
PI ≤ 7 and DI > 1.5	9	2	7
PI > 7 and DI ≤ 1.5	22	14	8
PI > 7 and DI > 1.5	10	3	7
PI > 7 and/or DI > 1.5	41	19 (46%)	22 (54%)

$\chi^2 = 12, P < 0.001$ (PI ≤ 7 and DI ≤ 1.5–PI > 7 and/or DI > 1.5).
DI = DNA index. PI = Partial index.

mind that as much as about 80% of patients with early cervical cancer are without lymph node metastases, the identification of these patients, who could be sufficiently treated with a less extensive operation, would considerably reduce the frequency of complications.

In the present study of 185 patients with early cervical cancer it was possible preoperatively to identify 65 patients, about 30%, without regional lymph node metastases, with a combination of flow cytometric DNA index and the partial index morphological grading system. The difference in metastatic frequency cannot be explained by different tumour size as there was no difference in the distribution of tumour size between the two groups (data not shown).

In previous studies a number of tumour features have been correlated with high incidence of nodal metastases in early cervical cancer, such as tumour size [14–17], depth of tumour invasion [16–20] and vascular invasion [17, 19, 21], whereas tumour type, degree of lymphocytoplasmocytic infiltration and borderline characteristics seem to be less constantly associated with lymph node metastases. These observations have not led to any alternations of the treatment modalities in early cancer of the cervix.

In previous studies it was shown that the score value of the MGS histopathological grading system was correlated with the survival of patients with carcinoma of the cervix [1, 2] and using a combination of the MGS score and the DNA index determined by flow cytometry it was possible to distinguish a group of patients with early cervical carcinoma who had very low risk of local metastases [5, 22].

Employment of the MGS score system is not without problems. It is time consuming and some of the eight morphological parameters may be difficult or even impossible to evaluate unless the biopsy specimens are large and of very good technical quality. These difficulties are partially avoided when the PI grading is used. PI consists of only four of the MGS parameters and has been shown to be almost as strong in prognostic prediction.

Table 4. Overall interobserver agreement and κ coefficient of PI grading before and after discussion of the grading criteria

	Before discussion	After discussion
Agreement	0.733	0.933
κ	0.594	0.722

PI = Partial index.

One of the major problems in the subclassification of patients with malignant diseases based on histomorphological grading is the considerable interpathologist variation as demonstrated in a number of both previous and recent reports [23–25]. The present study of the interobserver variation of the PI showed, however, that if the grading criteria were discussed in common between the analysis of two different series of biopsy specimens, the agreement rate increased from "moderate" (κ coefficient = 0.594) to "substantial" (κ coefficient = 0.722). Therefore, after mutual agreement of the grading criteria, and probably followed by periodical refresher courses, it is possible to obtain a reasonable reproducibility in tumour grading.

Thus, from the present investigation it can be concluded that by means of a combination of flow cytometric DNA analysis and determination of the PI score in preoperative biopsy specimens from patients with early cervical cancer, it is possible to reliably select a group with very little risk of lymph node metastases. It is, therefore, suggested that such patients could be sufficiently treated with a less radical operative procedure and most importantly, without lymphadenectomy.

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Eur J Cancer, Vol. 29A, No. 3, pp. 340–342, 1993.
Printed in Great Britain

0964–1947/93 $6.00 + 0.00

Positive Results of Adjuvant Mitomycin-C in Resected Gastric Cancer: A Randomised Trial on 134 Patients

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In order to evaluate the results on successful adjuvant chemotherapy in resected gastric cancer we performed a randomised trial on 134 patients in two arms: a control one with no further treatment after surgery versus a treatment arm given mitomycin-C (MMC), 20 mg/m² intravenously one day every 6 weeks for four courses, starting before the sixth week after surgery. The median follow-up was 105 months. In the control arm, 49 out of 66 patients died due to recurrence, versus 40 out of 68 patients in treatment arm. Actuarial survival curve was statistically significant ($P < 0.025$) in favour of the treatment group. Liver metastases were lower in adjuvant group than in the control group (8/68 versus 19/66). Toxicity was mild. Main toxic effects were thrombocytopenia, leukopenia, nausea and vomiting. A pelvis renal cancer as a second malignancy 8 years after gastric cancer was observed. In that particular case MMC was given after surgery. We conclude that adjuvant chemotherapy based on MMC given in the early period after surgery, improves survival rate in gastric cancer resected patients.
Eur J Cancer, Vol. 29A, No. 3, pp. 340–342, 1993.

INTRODUCTION

GASTRIC CANCER has a poor prognosis, even in patients undergoing surgery. In a review of 1479 cases, Dupont *et al.* [1], observed that 50% of resected patients died within 2 years of "curative" surgery. In Japan, in the past 25 years there has been a progressive improvement in the cure rate and long term survivors among patients with gastric cancer [2]. The reason for this improved survival has been the emphasis on early diagnosis, on extensive surgical procedures and because of the routine use of adjuvant chemotherapy. For this reason we initiated a randomised trial with adjuvant mitomycin-C (MMC) preliminary results of which have been previously published [3]. The life-table probabilities of survival were significantly improved in the treatment arm relative to the control arm after 5 years of follow-up and persists after 10 years [4]. We have now continued the case entry up to 134.

We report the long term follow-up results in all patients.

PATIENTS AND METHODS

Between 1977 and 1983, 134 consecutive patients were recruited to a randomised clinical trial with two arms: first, a control arm given no further treatment after resected surgery; secondly, a treatment arm, given mitomycin-C (MMC), 20 mg/m² intravenously once every 6 weeks for four courses. The inclusion criteria consisted of a histological diagnosis of gastric adenocarcinoma in one of the following categories (according to the UICC staging system; Ref. 5): T1 either with

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Received 24 Feb. 1992; accepted 8 July 1992.