

Prognostic Significance of DNA Content and Nuclear Morphology in Borderline Ovarian Tumors

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Received June 22, 1992

We used the technique of image analysis to simultaneously measure DNA content and nuclear morphology of 21 borderline ovarian tumors. Aneuploidy was identified in 9 of 21 tumors and was unrelated to tumor stage or nuclear grade. Morphometric nuclear features that were measured included size, shape, texture, and average density. Nuclear size and shape were positively correlated ($r = 0.507$), and nuclear size and average density were negatively correlated ($r = -0.772$). Six tumors recurred and recurrence was significantly associated with tumor aneuploidy ($P = 0.046$), stage III tumors ($P = 0.03$), and increased nuclear texture ($P = 0.07$). These results suggest that measurement of DNA ploidy and nuclear morphology using image analysis can provide important prognostic information in patients with borderline ovarian tumors. © 1993 Academic Press, Inc.

INTRODUCTION

Borderline ovarian tumors (BOT) account for 14–16% of all ovarian epithelial neoplasms. Although most patients with BOT can be cured by surgical excision, approximately 15% develop tumor recurrence and die from their disease [1–4]. Tumor recurrence has been associated with advanced tumor stage [1,2,4,5], however, many patients with advanced lesions suffer no tumor relapse while other patients with early stage BOT develop tumor recurrence. The identification of patients most likely to suffer recurrence after primary surgical therapy remains an important clinical problem. Investigations into the relationship between tumor recurrence and histologic subtype, cytologic atypia, and invasiveness of extraovarian implants have provided inconsistent results [2,5–8]. These inconsistencies may, in part, be secondary to difficulty with the reproducibility of these qualitative parameters.

Techniques which quantify morphologic features of tu-

mor cell nuclei have been useful in predicting the biologic behavior of many types of invasive malignancies. Measurement of DNA content, or ploidy, has been shown to predict tumor relapse and survival in breast, bladder, prostate, and invasive ovarian cancers [9–15]. Morphometric features such as nuclear area and density and epithelial stromal relationships have been correlated with tumor recurrence and survival in breast, ovarian, and genitourinary cancers [16–19]. Studies of BOT have been limited by small patient numbers, yet suggest that both DNA ploidy and nuclear morphology may be prognostically important [20–23]. We undertook this study to further evaluate the prognostic significance of quantitative assessment of DNA content and nuclear morphology in BOT. We analyzed our tumor specimens using image analysis which allowed the simultaneous measurement of these features.

MATERIALS AND METHODS

Patients to be included in this study were identified by review of the University of Michigan Medical Center gynecologic tumor conference records from 1970–1988. Of 71 patients with BOT, 21 patients were selected based on the availability of pathologic material and a minimum clinical follow-up of 3 years.

Hematoxylin and eosin stained sections from each primary tumor were reviewed to confirm the diagnosis of BOT and to select representative areas for image analysis. Additionally, tumors were classified according to the degree of cytologic atypia using previously reported criteria [5]. Unstained 5- μ m thick paraffin sections were prepared for feulgen staining [24]. Stained specimens were then analyzed for DNA content and morphometric nuclear characteristics using a CAS 100 image analysis system (Cell Analysis System, Chicago, IL). This system consists

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of an IBM AT PC with an image processing board and a 20-MB hard drive, a digital video camera mounted on a standard microscope, and a high-resolution color monitor. The system software automatically selects and outlines nuclear profiles from each manually selected microscopic field. The investigator can modify and reject machine-selected nuclei or manually select additional nuclei. With each nucleus the image analyzer measures the size (perimeter), shape (perimeter²/area), average optical density, and texture (standard deviation of the optical density). DNA content was calculated as the integrated (total) optical density within each nuclear boundary. Data from each tumor was generated from at least 120 epithelial nuclei and 50 lymphocyte nuclei which served as internal diploid control. DNA content was reported as the DNA index (DI), a value calculated for each tumor by dividing the optical density measurement of the tumor cell G0/G1 peak by the optical density measurement of the lymphocyte G0/G1 peak. Aneuploidy was defined as a DI > 1.2 [25] or DI ≤ 1.2 if tumors had additional DNA peaks other than G2M [26] (Fig. 1). All histograms were reviewed without knowledge of clinical outcome.

The clinical records, operative notes, and pathology reports were reviewed to determine tumor stage, adequacy of staging operation, treatment delivered, and clinical outcome. Disease recurrence was defined as pathologically documented disease identified by clinical exam.

Associations between tumor ploidy, stage, grade, and recurrence were analyzed using Fisher's exact test for 2 × 2 contingency tables. The relationships among different morphometric parameters were assessed using linear regression analysis, and the association between the morphometric parameters and tumor recurrence was analyzed using mean values of each variable as covariates in a stepwise logistic regression.

RESULTS

Fifteen patients had tumors confined to the pelvis and 6 patients had advanced-stage tumors (Table 1). Staging surgery for all patients included total abdominal hysterectomy and bilateral salpingo-oophorectomy and palpation of upper abdominal contents. Fourteen patients also underwent omentectomy and peritoneal biopsies. Only 1 patient underwent lymph node sampling. Postoperative therapy was given to 5 of 6 patients with stage III tumors and 4 of 15 patients with stage I or II tumors. Postoperative chemotherapy included combination therapy in 5 patients and single-agent therapy in 2 patients. Pelvic radiation therapy was given to 1 patient with a stage Ib tumor while one patient with stage III disease was treated with postoperative whole abdominal radiation therapy.

DNA content analysis identified 12 diploid and 9 aneuploid tumors. Aneuploidy was unrelated to tumor stage

(4 of 6 stage III vs 5 of 15 stages I and II; $P = 0.33$) or histologic grade (2 of 5 grade III vs 6 of 14 grades I and II; $P = 1.0$).

Six tumors recurred with a mean time to recurrence of 39 months. Recurrence was significantly associated with advanced stage (4 of 6 stage III vs 2 of 15 stages I and II; $P = 0.03$) and aneuploid tumors (5 of 9 aneuploid vs 1 of 12 diploid; $P = 0.046$) but unrelated to tumor grade (1 of 5 grade III vs 4 of 14 grades I and II; $P = 1.0$). Of the four morphometric parameters, only texture was predictive of relapse ($P = 0.07$, Table 2). The morphometric features may not be independent. Nuclear size and shape were strongly positively correlated ($r = 0.507$) and nuclear size and average density were strongly negatively correlated ($r = -0.772$). Three of the six patients with recurrent tumor have died of their disease; two of these had aneuploid tumors.

DISCUSSION

We used image analysis to measure the DNA content and morphometric nuclear features of 21 BOT. The aneuploid rate of 42.8% identified in our series is consistent with, although slightly greater than, aneuploid rates of 21–36% reported in other series of BOT when DNA content was measured using image analysis [21,23,27,28]. In contrast, lower rates of aneuploidy in BOT (0–12.5%) have been reported in most series that employed flow cytometric techniques to evaluate ploidy [29–31]. Image analysis can analyze a limited number of individually selected tumor cell nuclei, whereas flow cytometric techniques analyze a much greater number of nuclei that are mechanically and chemically dissociated from a tumor sample. The exact source of the nuclei analyzed (e.g., epithelium vs stroma, malignant vs benign) cannot be determined using standard flow cytometric techniques. Although previous studies comparing DNA ploidy measured by image analysis and flow cytometry have demonstrated excellent correlation in breast cancer cells [32], it is possible that the often focal nature of epithelial proliferations and nuclear atypism in BOT may result in discrepancies between these techniques. A prospective study comparing these two techniques would be useful in explaining such discrepancies.

Previous studies have suggested that DNA ploidy is prognostically important in BOT. Dietel *et al.* [21] noted six of seven relapses in patients with aneuploid tumors while Karen *et al.* [20] and Padberg *et al.* [23] have correlated DNA aneuploidy with increased tumor recurrence and shortened patient survival. In the present series aneuploidy was significantly associated with tumor recurrence and two of three fatal tumors were aneuploid. There was no relationship between DNA ploidy and nuclear grade or tumor stage although a correlation between advanced

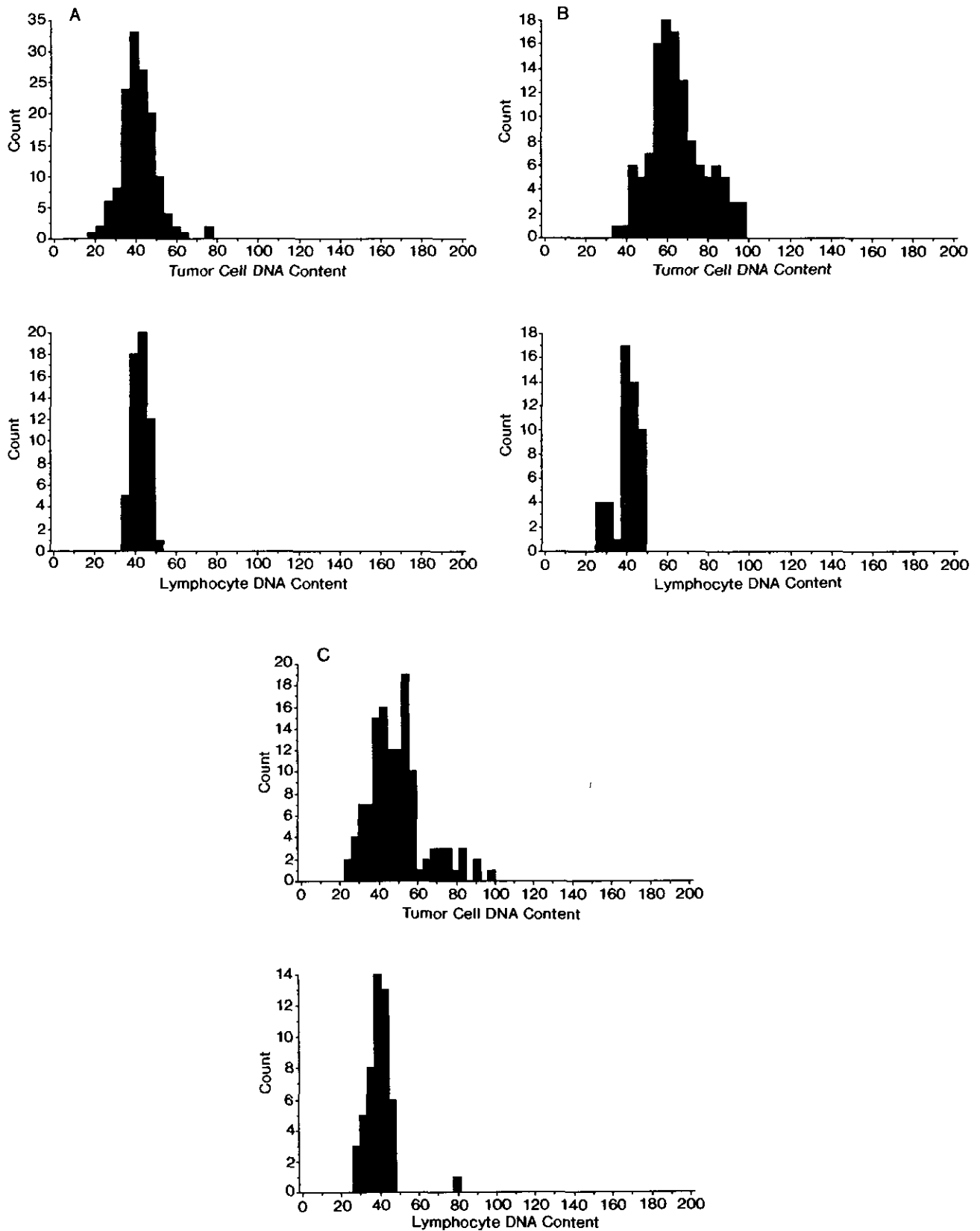


FIG. 1. Representative DNA histograms. (A) Diploid tumor; $DI = 1.0$. (B) Aneuploid tumor; $DI = 1.51$. (C) Aneuploid tumor; $DI = 1.05$ with additional tumor cell DNA peaks.

TABLE 1
Clinical Features of Tumors

Stage:	I	II	III
<i>n</i>	13	2	6
Histology:	Serous	Mucinous	Endometrioid
<i>n</i>	17	3	1
Therapy:	Surgery	Surgery— chemo	Surgery— radiation
<i>n</i>	12	7	2

tumor stage and aneuploidy has been reported by others [27,29]. We did note more recurrences with advanced tumors. We were unable to perform multivariate analysis to evaluate the relative prognostic importance of DNA ploidy and tumor stage because of our sample size. Reports from larger series [20,23] have suggested that ploidy is a superior prognostic factor.

The prognostic importance of quantitative nuclear morphometry has been demonstrated in carcinomas of the breast, urinary bladder, and ovary. In 49 patients with invasive ovarian cancer, survival was related to nuclear area and density but unrelated to nuclear shape [18]. Hytioglou *et al.* [35] demonstrated the discriminatory value of nuclear size for BOT and invasive ovarian carcinomas. He reported significantly smaller mean nuclear area and less variation in nuclear area measurements in BOT compared to invasive ovarian cancers. Baak *et al.* [17] analyzed 33 cases of BOT and demonstrated increased tumor recurrence with increased mitotic activity index and volume percentage of epithelium, features we were unable to measure using our system. Of the nuclear features we measured, texture was the only variable predictive of relapse. Texture is a measurement of chromatin distribution and increased texture in recurrent tumors reflects a greater degree of chromatin irregularity. The possibility of the other measured variables being prognostically important cannot be excluded with our sample size and future studies will continue to focus on these parameters. The variables measured in this study do not appear to be completely independent as correlation was noted between nuclear size, shape, and average density. A neg-

ative correlation between nuclear size and density in ovarian cancer cells has been previously reported [18].

The results of our study demonstrate that the measurement of DNA content and morphometric nuclear features using image analysis can provide prognostic information in patients with borderline ovarian tumors. Additional studies are needed to clarify the relative prognostic importance of these features.

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TABLE 2
Summary of Morphometric Data

Variable	Recurrent tumors (means ± SD)	Nonrecurrent tumors (means ± SD)	Logistic regression <i>P</i> value
Texture	0.061 ± 0.041	0.052 ± 0.007	0.07
Size (μm)	40.293 ± 13.995	33.293 ± 8.295	n.s.
Shape	13.313 ± 1.466	17.389 ± 2.106	n.s.
Density	0.240 ± 0.059	0.241 ± 0.041	n.s.

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