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Application of New Ploidy-related Parameters for the Diagnosis of Ovarian Tumours

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Abstract. *Differential diagnostics of borderline ovarian tumours and ovarian carcinomas is generally based on morphological criteria, which are not always sufficient for final diagnosis. Therefore, we investigated the practical diagnostic application of the CAS200 image analyzer and new ploidy-related parameters in a series of 68 borderline tumours and 42 low-grade carcinomas of the ovary. Highly significant differences between borderline and malignant lesions were found for the percentage of diploid cells (p=0.0001), the percentage of aneuploid cells between 4c and 8c (p=0.0001), the percentage of octaploid cells (p=0.0001), as well as for the 5c exceeding rate (p=0.0001). The difference concerning the ratio of tetraploid cells also reached the level of significance (p=0.0320). We suggest that new ploidy-related parameters evaluated by the CAS200 image analyzer can be helpful in ovarian lesions with unclear morphology.*

Borderline ovarian tumours show some of the features associated with carcinomas (nuclear atypia, high mitotic activity, stratification, glandular complexity, branching papillary fonds), but they lack definite stroma invasion (1-3). Some authors regard these tumours as low-grade serous adenocarcinomas with obviously better prognosis. They make up about 15% of all ovarian tumours. The differentiation between borderline and malignant ovarian neoplasms is not easy. Besides the known differences in nuclear features, morphometrical and image analyses can be of value for differentiation between borderline and malignant ovarian tumours (4-6).

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The CAS200 image analyzer makes possible the measurement of additional parameters defining single subfractions of cells, as for example the ratios of diploid, aneuploid, tetraploid, octaploid and 16-ploid cells. The prognostic significance of these parameters for malignant melanomas and malignant fibrous histiocytomas has been demonstrated (7-9). The main objective of this study was to check the practical application of new ploidy-related parameters for the diagnosis of ovarian tumours.

Materials and Methods

The material investigated consisted of 68 borderline tumours and 42 low-grade carcinomas of the ovary. The female patients' age ranged between 26 and 84 years, averaging 63 years.

The specimens were cut into 5-μm-thick paraffin sections, placed in 5N hydrochloric acid for 60 minutes and then in Feulgen stain for 1hour (CAS DNA staining kit by Cell Analysis System, Pharmingen--Becton-Dickinson, Hamburg, Germany). Thereafter, the slides were rinsed in acid alcohol, washed in xylol and covered with synthetic medium. Rat hepatocytes stained with Feulgen served as control cells. The slides prepared in this way were evaluated in a CAS-200 image analyzer (Becton-Dickinson) with Quantitative Analysis Software (QDA). The DNA content was quantified by assigning an optical density for each nucleus. The imaging system was calibrated by measuring the DNA content of rat hepatocytes. Ploidy analysis involved the measurment of 100-200 tumour cells with regard to the following parameters:

a) percentage of diploid cells (DNA index 0.83-1.22),
b) percentage of aneuploid cells 2c-4c (DNA index 1.23-1.83),
c) percentage of tetraploid cells (DNA index 1.83-2.22),
d) percentage of aneuploid cells 4c-8c (DNA index 2.23-3.84),
e) percentage of octaploid cells (DNA index 3.84-4.22),
f) 5c exceeding rate defined as percentage of cells with a DNA content exceeding 5c.

All DNA-histograms were classified according to the Auer scheme (10).

The differences between borderline lesions and carcinomas concerning the above-mentioned parameters were calculated using the SAS system and the Mann-Whitney *U*-test.

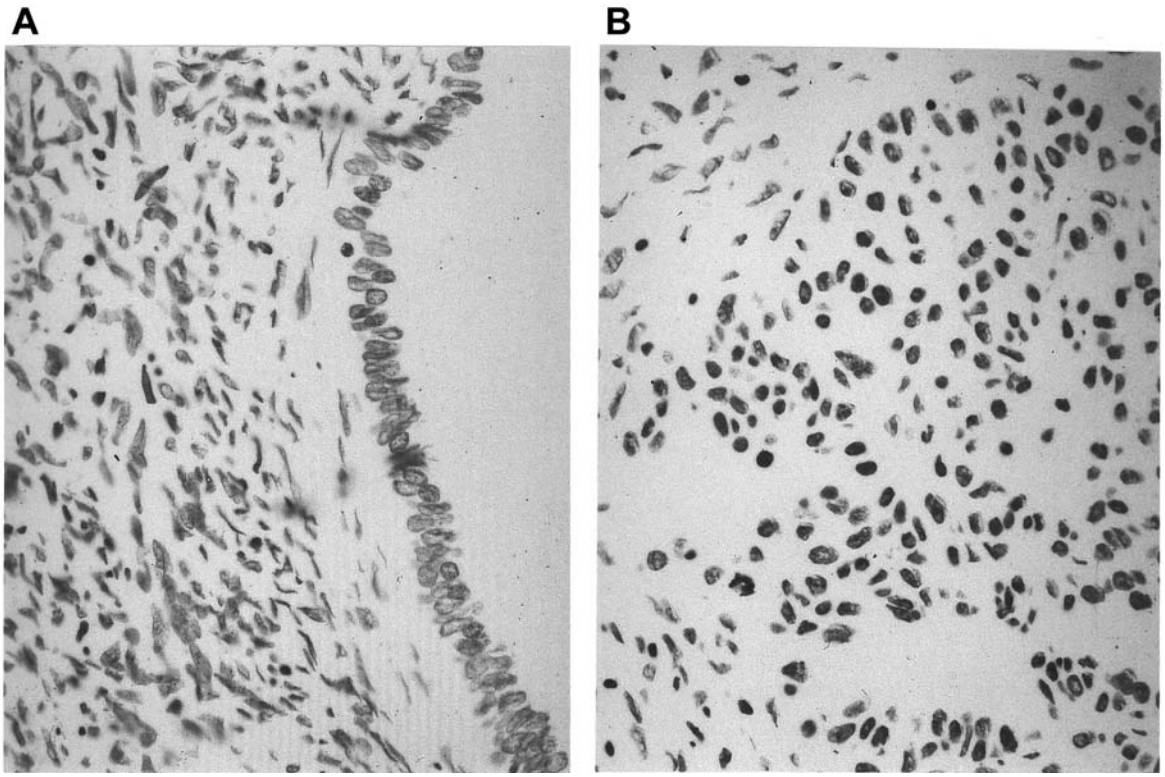

Figure 1. *A. Feulgen-stained borderline tumor of the ovary. B. Feulgen-stained low-grade ovarian carcinoma.*

Table I. *Auer histogram types in groups investigated.*

Auer type	Borderline lesions	Carcinomas
I	3/68	0/42
II	41/68	11/42
III	19/68	24/42
IV	5/68	7/42

Results

Classification of ploidy status according to Auer. In the group of borderline ovarian tumours, all 4 histogram types according to Auer were found. Most cases represented euploid Auer I- 3/68 and Auer II-41/68 histograms. Of the others, 19/68 were conspiciously (Auer III) and 5/68 clearly (Auer IV) aneuploid (Figures 1-2, Table I).

In the group of low-grade ovarian carcinomas, the Auer type I histogram was not found. Eleven out of 42 cases demonstrated type II histogram according to Auer. Twenty-four out of 42 lesions were conspicuously (Auer III) and 7/42 clearly (Auer IV) aneuploid (Figures 1-2, Table I).

Ploidy-related parameters. In the group of borderline lesions of the ovary, the percentage of diploid cells ranged between 5.56 and 86%, averaging 38.52%. The ranges of aneuploid cells between 2c and 4c oscillated between 0 and 58.72%, with mean reaching 29.35%. The ratio of tetraploid cells reached values between 0 and 50%, averaging 9.73%. The percentage of aneuploid cells between 4c and 8c ranged between 0 and 32.5%, averaging 2.84%. Octaploid cells in borderline lesions of the ovary were not found. The 5c-exceeding rate demonstrated values between 0 and 32.5%, averaging 2.84% (Table II).

In the group of low-grade carcinomas, the percentage of diploid cells ranged between 6.93 and 57.01%, averaging 25.24%. The ratio of aneuploid cells between 2c and 4c oscillated between 6 and 51.43%, averaging 30.09%. The percentage of tetraploid cells ranged between 0 and 29.7%, averaging 11.97%. The percentage of aneuploid cells between 4c and 8c reached values between 0 and 38%, averaging 13.94%. The ratio of octaploid cells ranged between 0 and 2.91%, averaging 0.75%. The 5c-exceeding rate oscilated between 0 and 40%, averaging 14.7% (Table III).

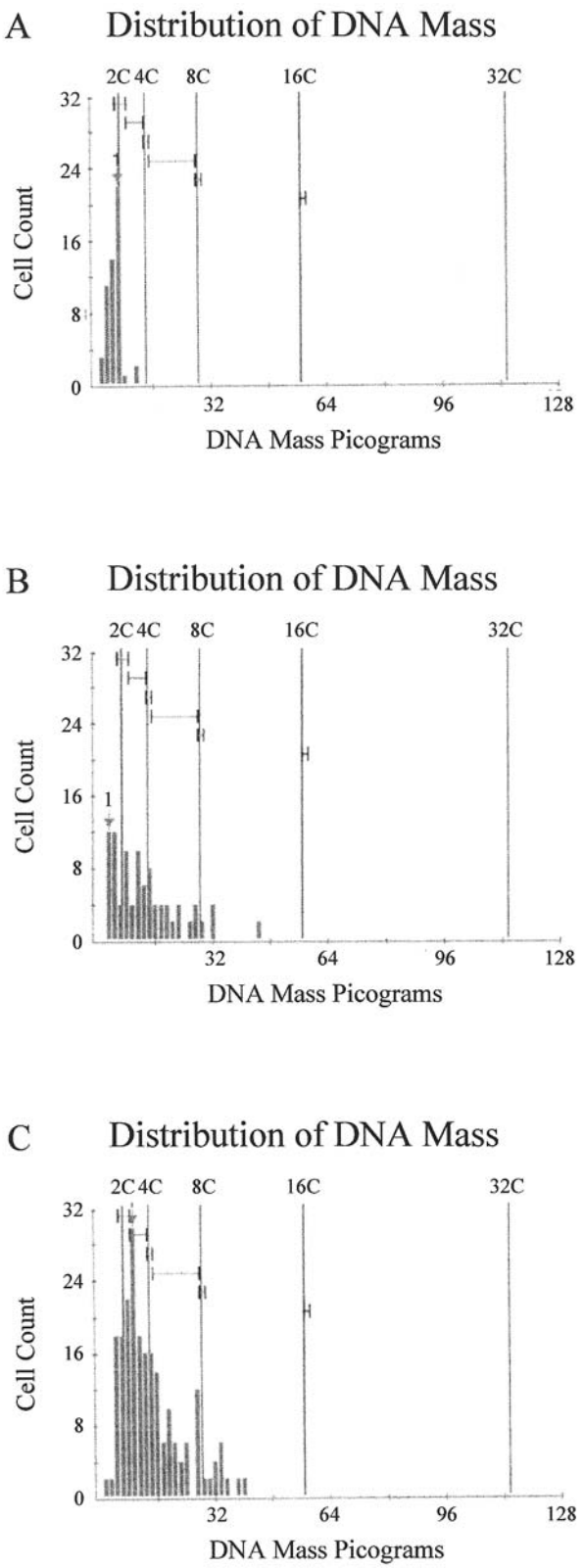

Figure 2. *A. DNA histogram type II acc. to Auer, euploid. B. DNA histogram type III acc. to Auer, suspiciously aneuploid. C. DNA histogram type IV acc. to Auer, clearly aneuploid.*

Table II. *Values of ploidy-related parameters in the borderline ovarian tumours.*

Parameter	Minimum	Mean	Median	Maximum
Diploid cells	5.56%	38.52%	33.65%	86.00%
Aneuploid cells 2c-4c	0.00%	29.35%	29.33%	58.72%
Tetraploid cells	0.00%	9.73%	7.74%	50.00%
Aneuploid cells 4c-8c	0.00%	2.84%	0.00%	32.50%
Octaploid cells	0.00%	0.00%	0.00%	0.00%
5cER	0.00%	2.84%	0.00%	32.50%

Table III. *Values of ploidy-related parameters in the low-grade ovarian carcinomas.*

Parameter	Minimum	Mean	Median	Maximum
Diploid cells	6.93%	25.24%	25.00%	57.01%
Aneuploid cells 2c-4c	6.00%	30.09%	31.13%	51.43%
Tetraploid cells	0.00%	11.97%	11.21%	29.70%
Aneuploid cells 4c-8c	0.00%	13.94%	12.00%	38.00%
Octaploid cells	0.00%	0.75%	0.88%	2.91%
5cER	0.00%	14.70%	13.00%	40.00%

Comparison of borderline and malignant tumours of the ovary. Highly significant differences between borderline and malignant lesions were found for the percentage of diploid cells ($p=0.0001$), the percentage of aneuploid cells between 4c and 8c ($p=0.0001$), the percentage of octaploid cells ($p=0.0001$), as well as for the 5c-exceeding rate ($p=0.0001$). The difference concerning the ratio of tetraploid cells also reached the level of significance ($p=0.0320$). The percentage of aneuploid cells 2c-4c did not differ significantly between the groups investigated ($p>0.05$).

Discussion

It has been shown, in a very large series of borderline ovarian lesions with long-term follow-up, that aneuploidy

status is clearly associated with poor prognosis. This fact was confirmed by several research groups. Aneuploidy status was reported to correlate with invasion in the peritoneal implant and with the microscopic appearance of the ovarian tumour. A clear guideline concerning the therapy scheme based on ploidy status in this groups of tumours, however, does not exist (11-13).

Generally, about 30-40% of borderline tumours are reported to be aneuploid. A similar aneuploidy ratio was found in our study. Some different aneuploidy ratios reported by other authors can only be explained by differences in techniques applied and tumour collections investigated.

In this study, highly significant differences between borderline and malignant tumours of the ovary were demonstrated not only concerning the 5c-exceeding rate, but also concerning the percentages of diploid cells, tetraploid cells, aneuploid cells between 4c and 8c, as well octaploid cells. In our opinion, application of these additional parameters is very useful in terms of differential diagnosis.

Normally, aneuploidy can be observed when the change of the total DNA content is sufficiently perturbed to be detected by image cytometry. Currently, aneuploidy is often used to describe cells in which the proportional number of each individual chromosome is incorrect if chromosomes are abnormal (14). This fact makes the aneuploidy status a sensitive tool in detecting genetic abnormalities not yet manifesting themselves morphologically, in the early stages of tumour progression, as we showed on the basis of borderline ovarian tumours.

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