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The Value of Morphometry to Predict Chemotherapy Response in Advanced Ovarian Cancer[1]

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SUMMARY

We evaluated the correlation of morphometric parameters to chemosensitivity. 63 patients with palliatively operated advanced epithelial ovarian cancer were investigated concerning their response to chemotherapy. Using multiple linear stepwise discriminant analysis of five morphometrical parameters 13 out of 17 responders and 37 out of 46 non-responders were correctly classified (76.5% sensitivity, 80.4% specificity, 79.4% efficiency). The five parameters were: nuclear area at the 10th percentile, standard deviation of the nuclear area, median value of the nuclear ovality, number of cells per area and mitotic activity index. To assess the performance of the discrimination formula when applied to new cases, the "leave one out" method was used. For our data the following corrected classification rates were obtained: 58.8% responders (10/17), 76.1% non-resonders (35/46) (efficiency 71.4%). Morphometry is a fast and reproducible method to objectively record a tumor's morphology. Our results indicate that there is a correlation between morphometrical features, response to chemotherapy and survival, which should be tested in further studies.

Introduction

The attempt of predicting the chemotherapy response in advanced epithelial ovarian cancer, by means of in vitro chemotherapy studies, has so far been of little success[1,16]. But morphological and morphometrical features have been successfully correlated to the patient's prognosis of survival[4,9,14], and recent results reveal a relation between morphometrical features and the response to chemotherapy[6]. There is an urgent demand for an exact and objective system of malignancy grading. This is of great importance for the patient, because the number of therapy concepts is increasing. Before initiating an aggressive therapy, which

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often leads to stress for the patient, it is vital to know the patient's chance of response. To investigate the relation between cell features and response to chemotherapy morphometry was chosen. This because it is a fast and objective low cost method suitable for routine work. Tumor mass reduction during chemotherapy was used as the criterion because it is of great value to know a patient's chance for debulking surgery after chemotherapy.

Material and Methods

The study was based on observations that tumor-morphology and -morphometry as well as the response to chemotherapy are correlated to survival[4,9,10,14,19]. In the light of this we investigated whether the degree of differentiation and the response to chemotherapy are correlated to each other.

Patients: The subjects for this retrospective study were patients who had been referred to the Oncological Department of the Salzburg General Hospital, Austria between 1978 and 1986. The criterion for inclusion was 1) a diagnosis of advanced epithelial ovarian cancer (FIGO III, IV) as confirmed by histological examination of laparatomy samples[17]. 2) No radical tumor resection was performed and residual tumor masses remained in the abdomen. The single tumor nodule was larger than 2 cm, which corresponds to the stage FIGO IIIc in the literature[5,15]. 3) All patients had received full-dosage adequate postoperative chemotherapy. From these patients all clinical and pathohistological data were available. 4) Patients were divided into two groups, responders and non-responders: responders were patients with a tumor mass reduction during chemotherapy of at least 30%, confirmed by repeated computer tomography or second-look operation. All other patients were classified as non-responders. 63 patients were included in the study, ranging in age from 29 to 83 years (mean 58). 17 patients were responders and 46 patients non-responders. The patient's data are listed in Table 1. There was no significant difference in the proportion of responders and non-responders when tumor stage, cell type and drug regimen were considered separately (p = 0.4, 0.8, 0.4, chi square test).

Table 1. Clinical data of 63 patients investigated

	patients		
	all n = 63	responders n = 17	non-responders n = 46
Figo[17]			
III	42	12	30
IV	21	5	16
Cell type			
Serous	52	15	37
Mucinous	2	0	2
Endometroid	0		
Mixed	5	1	4
Undifferentiate	4	1	3
Borders grade of malignancy[14]			
I	4	3	1
II	9	3	6
III	21	4	17
IV	29	7	22
Regimen			
FC	35	10	25
FCA	6	1	5
AC	17	4	13
CAD	5	2	3

F = 5-Fluoruracil, C = Cyclophosphamide, A = Doxorubicin (Adriamycin), D = Cisplatin.

Table 2. Definition of morphometrical parameters

Nuclear area, expressed as (1.) median area (P 50), (2.*) the area at the 10th (P 10) and (3.) the 90th (P 90) percentile, (4.) the mean area, (5.*) the standard deviation of the nuclear area, (6.) the % of nuclei exceeding the double median area and (7.) the % nuclei not exceeding the half median area.
Nuclear ovality, defined as the ratio of minimum and maximum diameter, expressed as (8.*) the median value (P 50), (9.) the 10th percentile (P 10) and (10.) the 90th percentile (P 90).
Nuclear irregularity of the perimeter, expressed as (11.) the median value (P 50) of the ratio between the nuclear perimeter and maximal diameter × minimal diameter, (12.) the ratio at the 10th percentile (P 10) and (13.) at the 90th percentile (P 90).
Nuclear irregularity of the area, expressed as (14.) the median value (P 50) of the ratio between the nuclear area and maximal diameter × minimal diameter, (15.) the ratio at the 10th percentile (P 10) and (16.) at the 90th percentile (P 90).
(17.*) Nuclear density, the number of nuclei divided by area parenchyma. (18.*) Mitotic activity index, the number of mitotic figures assessed in ten fields at a magnification of 400× (40× planapo objective, with 0.75 numerical aperture) with the diameter of each field being 450 micrometer.

* parameters that finally were entered into the MLDA.

Morphometry: Eighteen morphometrical parameters were investigated, described in Table 2: all parameters except the mitotic activity index were measured by using an interactive digital image analyser system (MOP 30, Zeiss Kontron): two photographs were analysed from the original haematoxylin eosin stained section (280 × 200 micrometer tumor areas magnified 1000×). The criteria for selecting the most appropriate tumor areas were these: highest cellularity, highest mitotic rate, strongest atypicality and avoidance of areas with inflammation or necrosis. The mitotic activity index was counted from the original hematoxylin eosin stained section (All parameters used are described in Table 2).

Statistical analysis: Two packages of statistical analysis were used (SPSS, SURVREG). In a first step all parameters exceeding a p-value of 0.2 in a Mann-Whitney-U test (responders against non-responders) were excluded from further analysis. For the remaining parameters correlation coefficients were computed and of each pair of parameters with a correlation coefficient > 0.95 the one with the lower p-value was retained for further analysis. If correlating parameters had almost equal p values the one that better contributed to the variance of parameters in the following multiple linear discriminant analysis was retained. The reduced set of parameters was entered into a multiple linear discriminant analysis (MLDA) with stepwise variable selection[11,12]. The procedure enters a variable only if it significantly improves the discrimination (p < = 0.05) at the present stage. To assess the performance of the discrimination formula when applied to new cases, the "leave one out" method was used[12]. Kaplan-Meier's method was used for the calculation of survival probability, log rank test for the comparison of survival in two groups (responders against non-responders)[13]. The Cox regression model was used to

investigate the impact of the morphometrical parameters on survival[8].

Results

Response and survival: The median survival time was 37 months for responders and 11 months for non-responders. The difference between responders and non-responders was highly significant ($p < 0.002$, log rank test) (Fig. 1).

Significance of single parameters to predict response: Of the eighteen morphometrical parameters investigated, eight had a p-value < 0.2, Mann-Whitney-U test of responders against non-responders (Table 3), and were further analysed. In this group of 8 parameters 3 parameters were highly correlated to 2 others (the mean and median nuclear area to the nuclear area at the 10th percentile, the nuclear area at the 90th percentile to the standard deviation of the nuclear area, correlation coefficient > 0.95). The mean and median nuclear areas were excluded due to a higher p value in the Mann-Whitney-U test. The nuclear area at the 90th percentile was excluded as the standard deviation of the nuclear area better contributed to the variance of parameters that were entered into the MLDA.

Discriminant analysis: The five remaining parameters, nuclear area at the 10th percentile, standard deviation of the nuclear area, median value of the nuclear ovality, nuclear density and mitotic activity index, were entered into a MLDA. All these parameters were accepted by the selection procedure ($p = 0.0013$). 76.5% (13/17) responders and 80.4% (37/46) non-responders were correctly classified (79.4% efficiency).

The discriminant function is given by:

$D = -0.0856 \times$ mitotic activity index $- 0.0459 \times$ nuclear area P 10
$+0.0671 \times$ nuclear area standard deviation
$+14.7 \times$ nuclear ovality P 50 $+ 397 \times$ nuclear density $- 12.2$.

Values of $D >= 0$ imply allocation to group responders, values < 0 allocation to group non-responders.

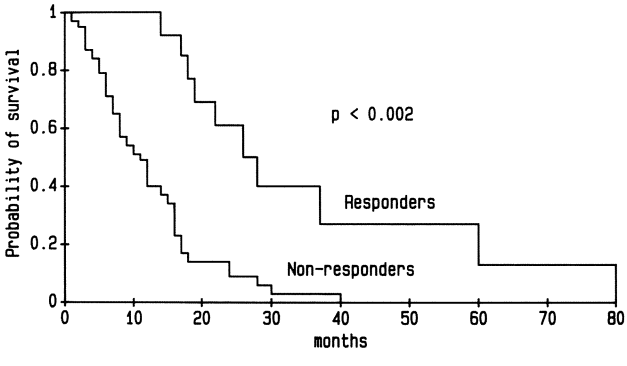

Fig. 1. Probability of survival for responders and non-responders. Calculated by Kaplan-Meier method, total n = 63, responders n = 17, non-responders n = 46. $p < 0.002$, log rank test.

Adding tumor stage, cell type or drug regimen subsequently did not significantly improve the discriminant efficiency.

Classification by leave one out method: 58.8% (10/17) responders and 76.1% (35/46) non-responders were correctly classified (71.4% efficiency).

Influence of morphometrical features on survival: The most significant prognosticators of survival were nuclear density ($p = 0.001$), nuclear ovality P 10 ($p = 0.01$) and the % nuclei not exceeding the half median area ($p = 0.04$) (Cox regression model).

Discussion

Modern concepts of tumor therapy demand an exact and objective system of malignancy grading[3]. Morphometry, reflecting objectively a tumor's morphology, is a fast and reproducible method whose predictive impact on the patient's prognosis of survival has been described[2,4,7]. In a pilot study we were able to show that morphometrical data were stronger discriminators than subjective histological grading and staging systems[18]. There the significance of the discriminating rule was improved by adding quantitative DNA data to morphometrical data. This is in agreement with Baak that the morphometrical as well as quantitative DNA data increase the prognostic power compared with traditional morphology[2–4]. The aim of the present study was to use parameters which are fast and easily measureable and could be introduced in the pathologist's routine work. Morphometry is both faster and cheaper than most other objective methods; quantitative DNA measurement, therefore, was not performed in our present study. Morphometry alone could discriminate responders with a 79.4% efficiency. The above classification results are probably too optimistic when the discrimination formula is applied to new cases, since the data set for deriving the formula (learning set) and that for testing its classification properties (testing set) were identical. Corrected estimates of the misclassification for new cases may be obtained using the "leave one out method": each case is classified using all the other cases, but not the case itself, to derive the discriminant function. For our data the following corrected classification rates were obtained: 71.4% efficiency, 58.8% sensitivity (10/17), 76.1% specificity (35/46).

In conclusion: our results indicate that nuclear features are not only correlated to survival but also to the response to chemotherapy. The clinical significance can be reached by discriminant analysis of several parameters. Further increase of the accuracy is perhaps possible by adding the quantitative DNA content, the volume % epithelium and the pattern recognition grading to morphometrical data[5,6].

The present study endeavors to provide answers to two clinical problems: if a patient's condition is critical and is not willing to consider chemotherapy, an analysis which would predict a high probability of response to chemotherapy might convince the patient to accept such therapy. If the discriminant analysis indicates an insufficient che-

Table 3. Mean values (mean), standard deviation (STD) for responders (R) and non-responders (NR) of the morphometrical variables with a p – value < 0.2 (Mann-Whitney-U test)

	R		NR		p-value
nuclear parameter	mean	STD	mean	STD	
*area P10	20.864	5.624	24.184	6.666	0.02
area P50	32.261	9.161	36.323	9.814	0.05
area P90	49.327	15.472	55.707	15.948	0.06
mean area	34.443	10.254	38.741	10.493	0.04
*area STD	12.327	5.414	13.940	5.233	0.09
*ovality P50	0.693	0.076	0.661	0.034	0.11
*density	0.0074688	0.0030907	0.0055230	0.0016624	0.04
*MAI	1.647	2.317	3.196	3.739	0.03

* Parameters that finally were entered into the MLDA. – STD = Standard deviation, MAI = Mitotic activity index, R = responders, NR = non-responders.

motherapy response, this might convince the clinician to choose a less stressful treatment. Thus clinicians and pathologists working together are faced with the challenge of selecting the best, most appropriate therapy for each individual patient.

The results should be looked upon as being a guide for further investigations.

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