Morphometric data to FIGO stage and histological type and grade for prognosis of ovarian tumours

J P A BAAK,* E C M WISSE-BREKELMANS,* F A LANGLEY,† A TALERMAN,‡
J F M DELEMARRE§

*From the *Pathology Institute, Free University Hospital, Amsterdam, The Netherlands, the †Department of Pathology, St Mary's Hospital, Whitworth Park, Manchester, England, the ‡Department of Pathology, University of Chicago, Chicago, United States, and the §Department of Pathology, Antoni van Leeuwenhoek Ziekenhuis, The Netherlands Cancer Institute, Amsterdam, The Netherlands*

SUMMARY The prognostic value of using histological typing, grading, and morphology, in addition to clinical staging, was assessed in 98 cases of invasive ovarian cancer of the common epithelial types (serous, mucinous, and endometrial). All of these cases had at least five years of follow up.

When regression analysis was used, the International Federation of Gynaecology and Obstetrics' (FIGO) staging system was the best indicator for prognosis. Analysis of a combination of morphometric features was the second best indicator, being especially useful for the those patients with stage I disease. Variables that indicated a relatively poor prognostic outcome were mitotic index above 30; volume percentage epithelium above 65%; shortest nuclear axis above a mean of 1.1 micrometres.

Histological typing of ovarian tumours was of limited value; mucinous tumours have a somewhat better prognosis than serous tumours, but the prognostic value of typing alone was found to be limited. Qualitative histological grading was useful, but the prognostic value of morphometric grading was better. Measurement of morphological features with an interactive computer program is simple and can be done by a pathologist or a technician: in future it is likely that such automated systems of measurement will improve the objectivity of tissue analysis.

Previous studies have shown that morphometry can be used to distinguish between serous and mucinous borderline and malignant ovarian tumours.^{1,2} Furthermore, quantitation of microscopical features can predict the outcome of patients with borderline lesions when the clinical staging and qualitative microscopical analysis have failed to do so.³ The strongest prognostic factors seemed to be the mitotic activity index and the volume percentage of epithelium. Morphometry of nuclear features was a far less useful prognostic indicator, although an assessment of nuclear shape had some predictive value—for example, there were more irregular nuclei in patients who did not survive five years postoperatively.

Whether morphometric techniques can also provide prognostically important information in frankly invasive serous and mucinous carcinomas of the ovary, which form the majority of all malignant ovarian tumours, as well as borderline tumours, remains a problem. This is especially important

because there are considerable prognostic differences in pathologists' typing and grading⁴ and therefore differences in predicted diagnosis.

More objective criteria are needed, and morphometry, because of its quantitative nature, can provide these.⁵⁻⁸

Better prognostic characterisation is important for refining treatment, especially in stage I cancers. Patients with only local tumour spread have a good prognosis, but 30% still die from metastases. Early systemic adjuvant chemotherapy may improve the prognosis, but the side effects can be severe, and it is therefore important to limit its use to patients with a poor prognosis.

Material and methods

Ninety eight patients with serous (n = 52), mucinous (n = 31), seromucinous (n = 5) or endometrioid (n = 10), and two cases of clear cell carcinoma of the ovary were studied. They were followed up for at least five years or until death; the percentage survival at

five years postoperatively was used as the criterion for assessment of prognosis.

The cases were obtained from the histology files in the laboratories where the authors work.

Clinical follow up was adequate, and both staging and sampling were performed according to standard criteria. Extensive staging was not performed. There were 46 stage I (47%), 12 stage II (12%), 38 stage III (39%) and two stage IV patients (2%). These figures are comparable with those in other series.⁹

The number of tissue blocks per tumour varied. In most cases one section per centimetre of tumour was available, stained with haematoxylin and eosin, the thickness of the paraffin section being 4 to 6 μ m.

The material used was obtained from a multicentre ovarian tumour study. Of the original 198 tumours in that study, 183 belonged to one of the common epithelial types, and follow up data were available in 161 of these. In 27 follow up was less than five years. Of the other 134 patients, five died from other causes, nine had unknown stage, and two tissue sections were of such poor quality that assessment was impossible. Twenty patients had borderline tumours. This left 98 patients with invasive carcinoma for analysis. Age, clinical stage, and microscopical characteristics of the tumours in this remaining group did not differ from those observed in the original group of 183 patients.

QUALITATIVE ANALYSIS

In a previous study all tumours had been graded by four of the authors (FAL, AT, JFMD and JPAB) on two occasions without prior knowledge about the stage, treatment, clinical outcome, or their own or each other's previous and present assessment of grade. The intraobserver and interobserver agreement varied from one grade to another and between pathologists.^{10 11} Therefore, the grade agreed on by at least two of us was used in the multivariate analysis to assess the correlation between the morphometric features and survival.

MORPHOMETRY AND REPRODUCIBILITY

Details of the morphometric techniques have been described in detail elsewhere.^{1 3} Briefly, a total of 14 quantitative features were investigated in each tumour on two occasions. The areas in which the measurements were performed, were carefully selected, on the basis of the following criteria: highest cellularity; highest mitotic rate; highest degree of cellular atypia; areas with inflammation, necrosis, or calcifications were avoided.

These criteria were reproducible among the different technicians after careful instruction. Twenty five randomly selected epithelial cell nuclei were photographed at a magnification of $\times 1000$, and the area, perimeter, shape factor (form ell = $4\pi \times$

area/perimeter²), longest axis, shortest axis, and nuclear axes ratio were measured. The mean and standard deviation of these features were calculated. The nuclear measurements were carried out on a commercially available graphic tablet coupled with a microcomputer (Mop-Videoplan, Kontron, Munich).

The proportions of epithelial and stromal tissue in the atypical areas were measured by a point counting technique⁵ using a 42 point grid (9.2×7.6 cm) placed on a projection microscope at $\times 200$ magnification. A minimum of 500 points were counted (if enough tissue was available).

The number of mitotic figures was assessed in 25 fields (mitotic activity index) at $\times 400$ magnification (objective $40\times$, numerical aperture 0.75), the diameter of each field being 450 μ m. Counts were started in the most cellular areas of the tumour. Adjacent fields were counted, but only those fields containing more than 50% epithelial tissue were used for these counts.

Recovery tests of all measurements showed a maximum inaccuracy of 5% of the mitotic activity index, the percentages of epithelial and stromal tissue, and the means of the nuclear features, both within and between different carefully instructed observers. The coefficient of variation of the SD of the nuclear features varied, but was usually higher than that of the means. Special attention was paid to the coefficient of variation of the SD of the shortest nuclear axis, which was 20%.

STATISTICAL ANALYSIS

Wilcoxon's rank sum test was used to determine significant differences between tumours from survivors and non-survivors. $P = 0.05$ was chosen as the limit of significance.

To find the microscopical feature or features with the highest correlation with survival multivariate linear stepwise regression analysis was performed with survival or non-survival as the dependent variable. Bivariate histograms were also made of the clinically important features. This approach was adopted to detect trends in unequal distribution, these not being easily detectable with the usual test for normal distribution of single features—Kolmogorov-Smirnov test—which was, however, also applied. In the stepwise regression analysis selection of a combination of features was done on the basis of the following criteria: the importance of the model; the increase of the squared correlation coefficient (RSQ) per step; the importance of the contributing features (in this order). Preference was given to the smallest combination of features.

Results

SINGLE VARIATE ANALYSIS

There were 98 patients with adequate follow up:

Table 1 Correlation between stage, tumour differentiation, and survival

Grade	Stage I		Higher stages	
	Percentage alive		Percentage alive	
Well differentiated	21	86	8	13
Moderately differentiated	12	67	22	14
Poorly differentiated	13	31	22	9
Total	46	65	52	12

36 (37%) had survived more than five years after the operation. Patients with a mucinous tumour had a somewhat better survival (42%) than those with a non-mucinous tumour (34%). The survivors were in an earlier stage of disease than the non-survivors; they also had better differentiated tumours (table 1).

Table 2 shows the morphometric features that were significantly different between the survivors and non-survivors. In spite of these differences there was considerable overlap (table 3).

Table 4 Features with strongest correlation with histological grade in stepwise regression analysis

Feature	R	p value
Step 1 Volume percentage epithelium	0.32	0.0001
Step 2 Volume percentage epithelium, SD of shortest nuclear axis	0.43	0.0001
Step 3 Volume percentage epithelium, SD of shortest nuclear axis Mitotic activity index	0.47	0.0001

MORPHOMETRY AND TUMOUR GRADE

Because of the strong relation between histological grade and prognosis, we investigated which feature, or combination of features, had the strongest correlation with the grade, using stepwise regression analysis: table 4 shows these features. The volume percentage epithelium, SD of the shortest nuclear axis, and the mitotic activity index were the features with the strongest correlation to histological grade.

Table 2 Differences in morphometric features between survivors and non-survivors at five year follow up*

Feature	Mean		p value
	Survivors	Non-survivors	
Volume percentage epithelium	73	80	0.0001
Mitotic activity index	33	58	0.0001
Mean nuclear perimeter	27.1	28.2	(0.06)
Standard deviation of:			
Nuclear perimeter	3.50	3.96	0.05
Nuclear area	12.67	14.75	(0.06)
Nuclear shortest axis	0.98	1.08	0.05

*Wilcoxon's test, two sided.

Table 3 Overlap in (significantly different) morphometric features

Feature	Mean (SD)	Minimum	Maximum
Volume percentage epithelium			
Survivors	73 (15.02)	47	91
Non-survivors	80 (12.63)	32	98
Mitotic activity index			
Survivors	33 (26.3)	0	117
Non-survivors	58 (44.3)	0	248
Mean nuclear perimeter			
Survivors	27.1 (3.78)	18.6	40.3
Non-survivors	28.2 (3.34)	18.4	35.1
SD nuclear perimeter			
Survivors	3.50 (1.04)	1.78	6.63
Non-survivors	3.96 (1.26)	1.83	8.70
SD nuclear area			
Survivors	12.67 (5.07)	5.64	30.59
Non-survivors	14.75 (6.56)	4.49	48.17
SD shortest nuclear axis			
Survivors	0.98 (0.23)	0.57	1.55
Non-survivors	1.08 (0.33)	0.59	2.00

Table 5 Features with strongest correlation with prognosis*

Feature	R	p value
Step 1		
Mitotic activity index	0.15	0.0001
Step 2		
Mitotic activity index, volume percentage epithelium	0.20	0.0001
Step 3		
Mitotic activity index, volume percentage epithelium, SD of shortest nuclear axis	0.23	0.0001

*Dependent variable five year survival, or not.

Assessing additional features did not give better results.

MORPHOMETRY AND PROGNOSIS

In a similar statistical analysis the prognostic value of the morphometric features was investigated. Survival or non-survival at five years was used as the dependent variable: table 5 shows the results. Note that compared with the results shown in table 4, more or less the same features were selected, but in a somewhat different sequence. Fig 1 shows the mitotic activity index and the volume percentage epithelium for the survivors and non-survivors. Patients with low values of these features generally have a good prognosis, whereas the outcome of those with higher values is much poorer. In agreement with the findings of earlier publications^{1,3} a mitotic activity index above 29 and a volume percentage of epithelium above 64 were associated with a worse prognosis. These thresholds resulted in three morphometric subsets of patients arbitrarily denoted as: A (mitotic activity index below 30 and percentage volume of epithelium at 65 or below); B (mitotic activity index below 30 and the volume percentage epithelium above 64); and C (mitotic activity index above 29). The mitotic activity

Fig 1 Mitotic activity index and volume percentage epithelium of survivors (open circles) and non-survivors (black stars).

Table 6 Five year survival of three morphometric subsets (features, mitotic activity index, volume percentage epithelium) for all cancers

	First assessment	Second assessment
Group A		
Mitotic activity index < 30	80% (n = 10)	73% (n = 11)
Volume percentage epithelium < 65		
Group B		
Mitotic activity index < 30	46% (n = 24)	50% (n = 22)
Volume percentage epithelium ≥ 65		
Group C		
Mitotic activity index ≥ 30	27% (n = 64)	26% (n = 65)

Table 7 Relation of prognosis with most significant combination of morphometric features in stage I patients only

Features and thresholds	No	No (%) patients alive
Mitotic activity index < 30	14	13 (93)
SD of shortest nuclear axis < 1.1 µm		
Mitotic activity index < 30	9	5 (55)
SD of shortest nuclear axis ≥ 1.1 µm		
Mitotic activity index ≥ 30	17	9 (53)
SD of shortest nuclear axis < 1.1 µm		
Mitotic activity index ≥ 30	6	3 (50)
SD of shortest nuclear axis ≥ 1.1 µm		
Total	46	30 (65)

index was the strongest prognostic factor. If the mitotic activity index is below 30, however, the volume percentage of epithelium can also predict prognosis: in such patients higher percentages of epithelium (above 64%) were associated with a worse prognosis (five year survival = 80% v 46%). In tumours with a

Fig 2 Mitotic activity index and standard deviation of the shortest nuclear axis in survivors (open circles) and non-survivors (black stars).

Table 8 Stage I cases, prognostic value of mitotic activity index and the percentage volume of epithelium*

Subsets and features	Total No	No (%) patients alive
(A) Mitotic activity index < 30 Volume percentage epithelium < 65	9	8 (89)
(B) Mitotic activity index < 30 Volume percentage epithelium ≥ 65	14	10 (71)
(C) Mitotic activity index ≥ 30	23	12 (52)
Total	46	30 (65)

*Thresholds used are the same as those used in table 6. Half of these patients fall into the morphometrically favourable subsets, compared with 35% for all cancers.

mitotic activity index above 29 the volume percentage epithelium was a less reliable prognostic factor.

The reproducibility of these three subsets was tested by blind duplicate assessments. Table 6 shows that the results were satisfactory.

MORPHOMETRY AND PROGNOSIS OF STAGE I PATIENTS ONLY

To see whether morphometry is of any value once the stage and the grade have been assessed the prognostic value of the morphometric subgroups was investigated in several subsets of patients. Assessment of the stage I patients separately (n = 46) pointed to the same morphometric features for prognosis as those already described. Once again, the mitotic activity index was the most important feature, followed by the standard deviation of the shortest nuclear axis SDSNA ($r = 0.25$; $p = 0.0001$). If the SDSNA was equal to or exceeded 1.1 micrometres the prognosis deteriorated (table 7, fig 2). The overall five year survival of these stage I patients was 65% (in agreement with the results of others). Table 8 shows the relation of the prognosis in stage I patients to the same morphometric features, which are, prognostically, most important for all the ovarian cancers (mitotic activity

index and volume percentage epithelium). The definitive general tendency of these stage I cases is to have lower values for mitotic activity index and volume percentage of epithelium. In only 50% of these stage I patients did the mitotic activity index exceed 29, in contrast to 65% of all ovarian cancers.

Table 9 shows the correlation between the FIGO stage, tumour grade, morphometry, and survival. Morphometry is of greater importance in stage I patients than subjective histological grading: in all grades the prognostic value of the three morphometric subsets was obvious. In stages II, III, and IV no definite conclusions could be drawn because of the small number of patients in each subset. It is of interest, however, that only one of these 52 patients fell into group A. Furthermore, in the moderately differentiated cancers group B patients had a higher survival than group C patients.

Discussion

In the present study the extent of the disease (stage) was the most important prognostic indicator, thus confirming the results of many other studies. Despite being in a good prognostic group several stage I patients died. When full clinical staging procedures are used, between 8–20% of supposedly stage I patients were found to have more extensive disease than was originally thought. Mortality five years postoperatively was between 25 and 40% (35% in this study). The discrepancy between these percentages indicates that extensive staging does not fully explain the mortalities for stage I patients. Other factors determine the prognosis as well, and the present study shows that morphometric characteristics are such factors. This is not surprising, many studies have shown the correlation between survival, nuclear and histological grade, and tumour type.¹² The problem with such qualitative features lies in the lack of consis-

Table 9 Correlation of stage, tumour grade, morphometry, and survival

Grade	Morphometric subset	Stage I		Higher stage	
		Total	Percentage alive	Total	Percentage alive
Well differentiated	A	6	100	1	0
	B	6	83	3	0
	C	9	78	4	25
Moderate differentiated	A	1	100		
	B	5	80	4	75
	C	6	50	18	0
Poorly differentiated	A	2	50		
	B	3	30	3	0
	C	8	25	19	5
Total		46		52	

Morphometry in ovarian tumours

tency and reproducibility of these assessments.^{10 11} Furthermore, depending on the observer, a high percentage of the cases are usually classed as grade 2 (intermediate; moderately differentiated), thus further reducing the practical value of grade and type.

The prognostic value of morphometry is rather important as it exceeds the value of subjective grading. Of the 16 stage I patients who died, 12 had a mitotic activity index over 30. In contrast, only five of the 23 patients with low morphometric values died (22%)—a figure comparable with patients with borderline tumours. Thus morphometry can help us to understand better the biological behaviour of ovarian tumours.

Should non-uniformly treated patients be used to evaluate prognostic factors? None of the survivors received adjuvant therapy; those who did died from recurrent disease. More aggressive multiple cytostatic drug regimens might have improved the survival figures, if the patients had remained disease free, but this was not the case. The survival figures therefore represent the "true" survival.

The question remains as to whether extensive staging and morphometry correlated with each other completely. If so, morphometry would be an extremely attractive alternative for extensive staging; inexpensive, easy, and without any risk for the patient. Yet it is more likely that these two prognostic factors (stage and morphometry) reflect different phenomena, as this was shown to be the case in breast cancer.¹³

Although the prognostic value of morphometry used in conjunction with staging is clear in the present study, it does not explain the mortality figures for all stage I patients: one third (five of 16) of the non-survivors fell into the intermediate risk ($n = 4$) or low risk ($n = 1$) categories. In contrast, 5% of the 41 patients with advanced disease and high mitotic activity were alive and well at five years. In the stage I cases sampling errors should be considered, but such an explanation is not valid for the stage III and IV patients who survived. Interestingly, using static and flow cytometry, prognosis was found to be correlated with abnormal DNA values.¹⁴⁻¹⁶ Perhaps a combination of morphometry and DNA cytometry would provide better prognostic information than either of these techniques alone. Such a study is currently under study in our laboratory. The number of endometrioid tumours in the present study was too small to evaluate the independent prognostic value of this histological type, but the prognostic value of the terms "serous" or "mucinous" was completely overshadowed by the morphometrical features.

Morphometry has the advantage of being inexpensive and technically simple, while standard material can be used. A disadvantage is that there is still a

subjective element. Repeated training, careful quality control, and standardisation of the measuring system as a whole is needed to guarantee good quality. Further automation with digital image processing will reduce the coefficients of variation to nearly zero. Pilot studies have shown the possibility of indentifying the mitoses automatically while disregarding most of the non-mitoses.¹⁷ Furthermore, epithelium and stroma could be distinguished and measured automatically. The correlation with manual measurements was extremely good in a few patients.¹⁸

Part of this work was carried out when Dr Baak and Mrs Wisse were at the Department of Pathology, SSDZ, Delft, The Netherlands.

This work was supported by grant No 28-834 from Praeventiefonds.

References

- 1 Baak JPA, Agrafojo Blanco A, Kurver PH, *et al*. Quantitation of borderline and malignant mucinous ovarian tumours. *Histopathology* 1981;5:353-60.
- 2 Baak JPA, Van der Ley G. Borderline or malignant ovarian tumour? A case report of decision making with morphometry. *J Clin Pathol* 1984;37:1110-13.
- 3 Baak JPA, Fox H, Langley FA, Buckley CH. The prognostic value of morphometry in ovarian epithelial tumours of borderline malignancy. *Int J Gynecol Pathol* 1985;4:186-91.
- 4 Baak JPA, Langley FA, Talermin A, Delemarre JFM. The prognostic variability of ovarian tumour grading by different pathologists. *Gynecol Oncol* (in press).
- 5 Weibel ER. *Stereological methods practical methods for biological morphometry* Vol 1. London: Academic Press, 1979.
- 6 Aherne WA, Dunnill MS. *Morphometry*. London: Edward Arnold, 1982.
- 7 Oberholzer M. *Morphometrie in der klinischen Pathologie*. Heidelberg: Springer Verlag, 1983.
- 8 Baak JPA, Oort J. *A manual of morphometry in diagnostic pathology*. Heidelberg: Springer Verlag, 1983.
- 9 Morrow CP. Malignant and borderline epithelial tumors of the ovary: clinical features, staging, diagnosis, intraoperative assessment and review of management. In: Coppleson M, ed. *Gynecologic oncology*. Edinburgh: Churchill Livingstone, 1981:655-79.
- 10 Baak JPA, Langley FA, Talermin A, Delemarre JFM. Disagreement in ovarian tumour grading and typing by different and the same pathologist. *Anal Quant Cytol* (in press).
- 11 Hernandez E, Bhagavan BS, Parmley TH, Rosenhein NB. Inter-observer variability in the interpretation of epithelial ovarian cancer. *Gynecol Oncol* 1984;17:117-23.
- 12 Dembo AJ, Bush RS, Brown TC. Clinicopathological correlates in ovarian cancer. *Bull Cancer (Paris)* 1982;69(3):292-7.
- 13 Baak PA, Van Dop H, Kurver PHJ, Hermans MA. The value of morphometry to classic prognosticators in breast cancer. *Cancer* 1985;56:374-82.
- 14 Erhardt K, Auer G, Bjorkholm E, *et al*. Prognostic significance of nuclear DNA content in serous ovarian tumors. *Cancer Res* 1984;44:2198-200.
- 15 Friedlander ML, Hedley DW, Taylor IW, Russel P, Coates AS, Tattersall MHN. Influence of cellular DNA content on survival in advanced ovarian cancer. *Cancer Res* 1984;44:397-400.
- 16 Hedley DW, Friedlander ML, Taylor IA. Application of flow cytometry to paraffin-embedded archival material for the study

- of aneuploidy and its clinical significance. *Cytometry* 1985;6:327-33.
- 17 Kaman EJ, Smeulders AWM, Verbeek PW, Young IT, Baak JPA. Image processing for mitoses in sections of breast cancer. A feasibility study. *Cytometry* 1984;5:244-9.
- 18 Schipper N, Smeulders AWM, Baak JPA, Young T. Staining evaluation and scene segmentation of ovarian tumors. *Pro-*

ceedings of the International Conference on Analytical Cytology 1985;11:438.

Requests for reprints to: Professor JPA Baak, Pathological Institute, Free University Hospital, De Boelelaan 1117, 1007 MB Amsterdam, The Netherlands.