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VOLUME CORRECTED MITOTIC INDEX (M/V INDEX) IN OVARIAN CANCER

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ABSTRACT

The M/V index expresses the mitotic activity of epithelial cancer as the number of mitotic figures per square millimeter of neoplastic epithelium in the microscope field. The M/V index is less subject to variation in the amount of neoplastic epithelium between neoplasms or in size of the microscope field than conventional mitotic index (mitoses/high power fields). In ovarian cancer, the mitotic activity had the best reproducibility among histoquantitative variables between different observers and laboratories. The M/V index was the best morphometric predictor in ovarian cancer in 105 cases studied. The prognostic value of two histological and two morphometric grading methods was inferior to that of the M/V index. Morphometric grading methods were better reproducible than subjective histological grading. In Cox's regression analysis the clinical stage (FIGO), the M/V index, and nuclear DNA content had independent prognostic value. Tumour ploidy emerged as the only independent predictor in advanced ovarian carcinoma. The M/V index was the best predictor of prognosis in stage I ovarian carcinoma.

Key words: mitotic index, morphometry, ovary carcinoma, static DNA cytometry.

INTRODUCTION

Histopathologists have traditionally used histological grading in the prognostication of malignant tumour types. Different approaches have been applied in the malignancy grading of ovarian tumours (Broders, 1926; Russell, 1979; Czernobilsky, 1984; Baak et al., 1986; Dauplat et al., 1988; Bichel et al., 1989). However, the World Health Organization classification of ovarian tumours (Serov et al.,1973) does not recommend any

grading method for use in the prognosis of invasive ovarian carcinomas. At the present time in histopathological practice, special effort is paid in the histological typing, but surprisingly often histological malignancy grading has been left undone. However, the rapidly developing modalities of ovarian cancer therapy have made demands upon the prediction of prognosis of the disease.

A good prognostic method measures biologically relevant features and is highly reproducible. The combination of these characteristics guarantees the effective prediction of prognosis (Collan, 1989). We have studied retrospectively these two qualities of morphometric methods in ovarian carcinoma and compared them with conventional histology, clinical features and with static DNA cytometry (Haapasalo et al., 1989a; 1989b; 1990a; 1990b; 1991a; 1991b). In this paper, the reproducibility and prognostic value of a new mitotic estimate, volume corrected mitotic index (M/V index) (Haapasalo et al., 1989a) will be reviewed. The material consisted of 105 invasive ovarian carcinomas sampled from 1956 to 1978 at the Mount Vernon Hospital, England (Atkin et al., 1979; Haapasalo et al., 1989b).

M/V INDEX

The basic idea in the volume corrected mitotic index is that in addition to the number of mitotic figures the area fraction covered by the neoplastic tissue (e.g. epithelium) is estimated in each high power field. This area fraction is a good estimate of the volume fraction (Vv) of the sectioned neoplastic tissue (Delesse principle) (Collan et al., 1983). If the mitotic number is divided by the area fraction of neoplastic tissue in the same microscopic field, the result will correspond to the mitotic estimate in a field completely filled with neoplastic tissue. If this estimate is divided by the area of the high power field (in mm^2), we will have an estimate which expresses the mitotic number per one square millimeter of neoplastic tissue. The M/V-index can be based on a number of microscope fields which has been considered appropriate to cover the needs for accuracy and representativity of the study.

The formula of the M/V index (M for mitosis and V for volume) (Haapasalo et al., 1989a) is

$$\text{Volume corrected mitotic index (M/V-index)} = k \left(\sum_{i=1}^{n} MI \right) / \left(\sum_{i=1}^{n} Vv \right)$$

where

n= the number of microscope fields studied

MI= number of mitotic figures of neoplastic epithelium in a microscope field selected randomly from the area of highest neoplastic cellularity. During the counting the microscope is focused once.

Vv= volume fraction of the neoplastic epithelium (in per cent) as estimated by the area fraction of the neoplastic epithelium in the microscope field. This is

estimated subjectively or with point-counting in the same field in which the mitotic count is made.

k = coefficient characterizing the microscope : $k = 100 / \pi r^2$, where r (in millimeters) is the radius of the circular microscope field.

As an example, in our microscope at 400x magnification $k = 100 / \pi(0.245)^2 = 530$. If there were 23 mitoses in 12 such fields and the corresponding sum of the volume fraction in the same fields was 890 , the formula would give

M/V-index = 530 x 23 / 890 = 14 mitoses / mm^2 of epithelium.

We have followed the recommendation of Baak and Oort (Baak et al., 1983) in identification of mitotic figures to distinguish them from pyknotic, hyperchromatic or deformed nuclei. The criteria for a mitotic figure were absence of nuclear membrane and presence of hairy instead of triangular or spiky nuclear projections, absence of clear zone in the center of the chromatin material, and basophilia instead of eosinophilia in the surrounding cytoplasm. Vv was estimated subjectively, but can also in practice easily be estimated with a point grid.

REPRODUCIBILITY OF M/V INDEX

The estimates of volume corrected mitotic index (M/V index) showed very good correlation when estimated from the same fields by two observers in the same laboratory (r= 0.995, Pearson product-moment correlation, N=46)(Haapasalo et al., 1990a). Repeat estimates from different fields by the same observer showed coefficient values of 0.939, and estimates by two different observers within one laboratory values of 0.936, respectively. The correlations between two independent observers in different laboratories was also good (r = 0.834). The level of reproducibility of the conventional mitotic activity index (MAI, mitoses/10 high power fields) was the same (r = 0.999, 0.949, 0.949, 0.894, respectively). The reproducibility of other morphometry (e.g. nuclear measurements by image analysis) was worse.

When comparing histological malignancy grading with morphometric grading between two observers working in the same institution (N=75) the morphometric malignancy grading appeared more reproducible (Haapasalo et al., 1990b). The percentages of agreement (kappa coefficients in brackets) for the histological grading methods of Czernobilsky (1984) and Russell (1979) and for the morphometric grading method of Baak (1986) were 74.6 % (0.59), 73.3 % (0.55) and 81.3 % (0.68), respectively. When the same tumours were divided into three groups of approximately the same size according to the M/V index (thresholds 10 and 20 mitoses / square millimeter of epithelium) the corresponding figures describing reproducibility between these groups were 80.0 % (0.70).

CORRELATION OF M/V INDEX TO OTHER PROGNOSTICATORS

The M/V index showed a significant difference between stage I ovarian carcinomas (means ± SD: 19 ± 19) and tumours in advanced stages (31 ± 29; p = 0.02, Mann- Whitney U-test, N=105)(Haapasalo et al., 1989a). The same figures for the mitotic activity index did not differ significantly (stage I: 22 ± 24, others : 31 ± 29; p=0.06). No difference existed in volume fraction estimates or morphometric nuclear measurements between stage I and stage II-IV cases.

Serous carcinomas showed the highest M/V index values (29 ± 23) and clear cell carcinomas the lowest (8 ± 6). When tumours were graded following the recommendations of Czernobilsky the estimates of the M/V index were at the lowest level in grade I tumours (n=27; 22 ± 21). The corresponding figures of grades II (n=56) and III (n=22) were 24 ± 28 and 33 ± 25, respectively. The difference in the values of the M/V index between grades was not significant.

The difference of M/V-indices between ploidy groups was significant when the carcinomas were divided into near-diploid and non-diploid tumours by static DNA cytometry and chromosome counts (mean±SD: 20 ± 21 and 29 ± 23, respectively; Mann-Whitney U-test: p = 0.05) (Haapasalo et al., 1991a). The mitotic activity index (MAI) failed to show a significant difference between ploidy groups.

PROGNOSTIC VALUE OF M/V INDEX

According to the value of each morphometric variable (mitotic indices, epithelial volume fraction estimates, nuclear measurements) the 105 patients were divided into three subclasses of approximately equal size (Haapasalo et al., 1989b). In the univariate analysis the M/V index and the mitotic activity index showed the greatest differences in terms of 5-year survival between different subclasses. The corresponding 5-year survival percentages for M/V index were 55 (M/V < 10), 28 (M/V 10 - 20) and seven (M/V > 20)(p= 0.00003, chi-square test) and for MAI 50 (MAI < 10), 34 (MAI 10 - 22) and seven (MAI > 20)(p= 0.0002). In 46 stage I tumours these indices showed even greater differences of survival: 82 - 87 % of patients with tumours having less than 10 mitoses/mm^2 of epithelium and 76 - 80 % of patients with tumours having less than 10 mitoses/10 HPF were alive at 5 years after the diagnosis of the disease, while 17 - 18 % of patients in high mitotic activity categories (M/V > 20 or MAI > 22) had survived. No significant differences in survival were found for other morphometric features.

When the prognostic sensitivity, specificity and efficiency (Galen et al., 1975) of the M/V index was evaluated with different thresholds, the index showed the best prognostic efficiency at a threshold of 10 mitoses/mm^2 of neoplastic epithelium. Here the 5-year survival of 75 - 79 % of patients could be correctly estimated (Haapasalo et al., 1990b). The

receiver-operating characteristic (ROC) curve showed that the M/V index regardless of the prognostic sensitivity/ specificity-level chosen, was generally superior to the malignancy grading methods of Russell, Czernobilsky and Baak in predictive power.
The prognostic value of morphometry and DNA cytometry was compared with conventional prognostic factors of ovarian carcinoma in a study of 91 ovarian carcinomas (Haapasalo et al., 1991b). All tumours with adequate morphometric, DNA cytometric and follow-up data are included. In univariate analysis, the clinical stage ($p<0.0001$), the M/V index ($p = 0.0004$), the mitotic activity index ($p=0.004$), grade of Baak ($p=0.008$),grade of Russell ($p=0.01$), cellular DNA content ($p=0.02$), histologic type ($p=0.02$), presence of ascites ($p=0.02$) and age of patient ($p=0.03$) proved to have prognostic value for 3-year survival. Of these, the stage ($p< 0.0001$), the M/V index ($p=0.0002$), MAI ($p=0.001$), the grade of Baak ($p=0.001$), the grade of Russell ($p=0.005$) and the histologic type ($p=0.01$) were associated with the 5-year survival.
The prognostic value of the M/V index is compared with the predictive power of modal DNA index in the same material of 91 carcinomas (Tbl 1). The threshold of M/V index is 10 mitoses/mm^2 of epithelium, the prognostically most efficient value of the index. The DNA index 1.3 is chosen for comparison, because this cut-off point has been shown to have the greatest predictive value in ovarian carcinoma in DNA cytometric studies (Klemi et al., 1989; Atkin, 1971). The predictive power is compared after each year of the 5-year follow-up. The prognostic power of the methods seems equal in the total material. However, the greatest prognostic difference is observed at three years of follow-up when the material is divided into two groups according to the DNA index. Later, at 4 - 5 years' follow-up, the same level of significance is reached by the M/V index. In stage I tumours only the M/V index of the two variables appears to have significant prognostic power. In advanced stages the small number of survivors makes the comparison of survival difficult, but it seems that the prognostic power of the DNA index in the total material is mainly due to the prognostic efficiency in advanced stages.
The results of the age-stratified multivariate analysis can be seen in Table 2. The stepwise Cox model shows that among the clinical, histological, morphometric and DNA cytometric predictors the most efficient prognostic indicators in the total material (N=91) are the clinical stage, M/V index and nuclear DNA content in sequence of decreasing importance. According to the Cox analysis of stage I tumours, the best prognostic predictors were the M/V index, the presence/absence of ascites and the cellular DNA content (Tbl 2). The variables here are in the order of significance, too, despite the larger coefficient seen with ascites, which is due to the division of this variable into two subsets, not three as in the case of the M/V index. In advanced stages (II-IV), only the cellular DNA content seems to have independent prognostic value.

Table 1. Survival (in per cent) according to the prognostically most efficient threshold values of the M/V-index and modal DNA index (DI) during the first years of follow-up. p-values are expressed when the difference between the number of survivors is significant (Chi-square test).

Years	N	1	2	3	4	5
All tumours						
M/V < 10	23	61	56 $_{0.01}$	52 $_{0.006}$	52 $_{0.001}$	52 $_{0.001}$
M/V ≥ 10	68	47	29	23	22	17
DI < 1.3	42	69 $_{0.01}$	52 $_{0.003}$	48 $_{0.001}$	45 $_{0.002}$	40 $_{0.005}$
DI ≥ 1.3	49	43	22	16	16	14
Stage I						
M/V < 10	11	91	91	91 $_{0.02}$	91 $_{0.01}$	91 $_{0.005}$
M/V ≥ 10	29	80	62	52	48	41
DI < 1.3	23	87	78	74	70	65
DI ≥ 1.3	17	77	59	47	47	41
Stages II-IV						
M/V < 10	12	33	25 $_{0.04}$	17	17	17 $_{0.01}$
M/V ≥ 10	39	33	5	3	3	0
DI < 1.3	19	47	21 $_{0.03}$	16 $_{0.02}$	16 $_{0.02}$	11
DI ≥ 1.3	32	25	3	0	0	0

Table 2. Results of the final Cox regression analysis. A = all tumours included; B = stage I tumours included, C= stage II-IV tumours included in the analysis. The variables are in the order of decreasing significance.

Variable	Exp(c)	Coeff.	SE	Chi-square	p
A. Clinical stage	2.14	0.760	0.140	35.8	0.000
M/V index	1.57	0.452	0.182	7.90	0.005
DNA content	1.29	0.257	0.140	3.50	0.061
B. M/V index	3.36	1.211	0.411	10.5	0.001
Presence of ascites	3.97	1.379	0.624	5.31	0.021
DNA content	1.56	0.444	0.273	2.90	0.089
C. DNA content	1.49	0.400	0.177	5.53	0.019

Exp(c) = Exp (coefficient); Coeff. = Coefficient; SE = standard error; p = p-value (enter limit $p < 0.10$)

DISCUSSION

One of aims in our studies has been the standardization of mitotic counting. The resulting index, the volume corrected mitotic index (M/V index) expresses the mitotic activity as the number of mitotic figures per square millimeter of neoplastic epithelium in the microscope fields (Haapasalo et al., 1989a). The M/V index will not be subject to the varying volume fractions of neoplastic tissue between different neoplasms. The variation of section thickness does not influence the result much because the microscope is focused only once before mitotic counting. With the M/V index, mitotic activity as measured by different microscopes (e.g. with high power fields of different size) can be compared easily and reliably. The M/V index is in linear relation with the number of mitotic figures per volume of neoplastic tissue (Collan, 1992).

Mitotic estimation was more reproducible than any of the other morphometric methods in our studies. In addition, malignancy grading by the M/V index and by the morphometric method of Baak appeared more reproducible than the histologic grading methods recommended by Czernobilsky and Russell (Haapasalo et al., 1990b). There was no significant difference in the reproducibility between the conventional mitotic activity index (MAI) and M/V index, but mitotic activity index (MAI) without area or volume correction showed better reproducibility between different laboratories (Haapasalo et al., 1990a). This can be understood easily: the M/V index uses two subjective numerical estimates, whereas the crude mitotic index uses only one. When estimating the M/V index, use of the point-counting method in the measurement of epithelial volume fraction instead of

subjective estimation might improve the reproducibility.
The M/V index was found to have greater prognostic value than the conventional mitotic activity index (MAI) among the 105 carcinomas studied (Haapasalo et al 1989b). This observation was confirmed separately in the early and advanced stages (Haapasalo et al., 1989b; 1991b). The mitotic counting for both these indices was performed in the same microscope fields. Thus, the difference in prognostic value is likely to be due to varying amounts of neoplastic epithelium in the microscope fields which biased the estimation of mitotic rate in the case of the mitotic activity index. The closer association of the M/V index with the clinical stage and the tumour ploidy (Haapasalo et al., 1991a) also suggests that the M/V index might be a biologically more relevant feature measuring aggressiveness of the neoplasms than the conventional mitotic activity index.
When comparing prognostic value of M/V index with DNA cytometry (Tbl 1), nuclear DNA content appeared to be prognostically efficient especially in advanced stages. Most of the patients with high DNA values will die during the first 3 years of follow-up, and this is especially true for advanced disease. However, in multivariate analysis (Tbl 2), morphometry (M/V index) seems to have even greater prognostic value than DNA cytometry, but - in our studies - only if early stages are included in the material studied. It was clearly shown in the present material that in stage I the most important prognostic information can be obtained by morphometry: the estimation of the proliferative activity of the tumour by the M/V index carries the most relevant prognostic information. The prognosis of ovarian cancer is determined to a large extent by the probability of metastatic dissemination (especially in stage I malignancy). Likewise, in breast cancer, the rate of proliferation seems to correlate with the probability of metastatic spread (Tubiana et al., 1989). Our results suggest that this is the case also in ovarian cancer. The cellular DNA content also seems to have some independent prognostic value in stage I, but our results and those of Baak et al. (1987) showed that DNA ploidy as a prognostic feature is overshadowed by the morphometric estimation of proliferative activity.
After the standardization of mitotic counting (the M/V index) problems still remain to be solved in the morphometric estimation of neoplastic proliferation. It is important that the tumour tissue is fixed immediately because a delay in fixation decreases the number of mitotic figures in histological specimens (Graem et al., 1979). After fixation, the sampling of tumour tissue for histology must be performed carefully. The peripheral parts of the tumour are preferred in sampling because autoradiographic studies on other neoplasms suggest that there is intratumoral variation in the proliferation activity, the proliferation being most active in the invasive border and periphery of tumour nodes (Rabes et al., 1985).
Two of the most difficult problems in the mitotic estimation are the identification of mitotic figures and the subjectivity in

choice of the areas where the mitotic evaluation has to be made. Sampling rules for choice of areas have been represented. For instance, Baak et al. used only fields containing more than 50 % epithelial tissue for mitotic counting (Baak et al.,1986). However, this rule caused problems in another study because enough epithelial tissue was not always present in the tumour specimen (Rodenburg et al.,1988). It seems that further international collaboration is necessary for the creation of pertinent sampling rules and to improve the reproducibility of mitotic estimation. Although our results suggest that after a long enough training period mitotic figures can be identified reliably (a very good intrafield reproducibility of mitotic counts)(Haapasalo et al., 1990a), modern markers of the cell cycle might be helpful in the identification of proliferating (e.g. anti-PCNA/cyclin) and dividing cells. The approach applied in the M/V index could readily be applied to evaluating such an improved immunohistologic proliferation index.

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