Editorial Article

Grading in ovarian cancer

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One of the complex problems of modern clinical oncology is establishing the proper prognosis of malignant tumors. Among the various parameters used for that purpose pathological grading remains the most important factor, though it is still a matter of controversy.

The above-mentioned controversy initially was begot mainly by imperfection of the methodical tools of pathology and their uncritical applications. In fact, the modern schemes of grading are based on the system of grading proposed by Broders in 1926 [1]. The percentage of differentiated and undifferentiated cells according to Broders reflects the malignancy of the neoplasm. Such an approach was a conceptual mistake. The morphology of cancer cells of course reflects their basic properties but one cannot equalize the cellular polymorphism (as Broders did) and biology of the tumour.

Concerning ovarian carcinoma (in this paper we focus on epithelial malignant tumours of the female gonad) some additional problems arise before the pathologist. They are connected with the specific morphology of the ovarian cancer representing the spectrum of heterogeneous neoplasms of different histogenesis and biological behavior. The difficulties in elaborating a unified system for grading ovarian carcinomas resulted in the descriptive character of all grading systems, which are too overburdened with details.

As an example we can quote in a simplified manner the grading system proposed by Russel [2]. Almost all nosological entities are graded using different principles, e.g. serous cancers are divided into four grades. Grade 1 serous carcinoma, which has almost exclusively a papillary pattern of epithelial growth. Even in diffusely invasive tumors the papillary pattern of growth is maintained. There are fewer than 2/10 HPF mitotic figures. Grade 2 serous carcinomas show a variety of histological patterns, although the cells are uniformly small with scanty cytoplasm and rounded nuclei. In over one third of the cases a papillary pattern predominates. In fewer cases a micropapillary pattern is dominant with complex branching and coalescence of micropapillary projections producing a lace-like picture. In other tumours the invading cells produce solid sheets, or nests of cells, without peripheral palisading. The least common pattern is glandular. Grade 2 serous carcinomas show fewer than 16/10 HPF mitoses. In Grade 3 serous carcinoma the predominant architectural pattern in 60% of tumours consists of large sheets of multiple small nests or morules of uniform small epithelial cells closely arranged in immature stroma. In 25% of Grade 3 tumours a lace-like pattern is dominant. In the remainder a more glandular appearance is most apparent. Mitotic activity is prominent and bizarre mitosis common. Grade 4 serous carcinoma is predominantly solid in pattern, with small areas being lace-like in appearance. No Grade 4 tumour shows papillary areas or calcification.

The grading system for mucinous carcinomas is more complex because of the marked mosaicism of their structure charcterized by coexistance of the foci of different grades of differentiation, even cancerous and benign tumours.

Practical application of such detailed systems of grading is rather inconvenient and their reproducibility is low, so the attempts to make the grading system for ovarian cancer more universal are quite logical. One of the attempts belongs to Shimizu *et al.* [3], who elaborated a system which includes three major parameters: architectural pattern, nuclear pleomorphism and mitotic count. These parameters are considered as independent and statistically significant prognostic variables for patients with primary ovarian carcinoma. Architec-

tural pattern includes glandular, papillary and solid growth. A predominant glandular, papillary or solid structure was assigned a score of 1, 2, 3 respectively. Final grade including the scores for nuclear polymorphism was allocated on the following basis: 3-5 points Grade 1 (well differentiated); 6-7 points Grade 2 (moderately differentiated) and 8-9 points Grade 3 (poorly differentiated). The main characteristic point of the new grading system assigns a glandular growth pattern a lower score than papillary. Mitotic activity is incorporated into the grading system (as independent from nuclear pleomorphism).

Some other grading systems [4] include not only the three parameters discussed, but additional factors such as a mode of invasion (well-defined and sharp borderline, less distinct borderline, diffuse growth), b) capsular penetration: 1. No penetration; 2. Tumour infiltration of the stroma close to the surface of the tumour, but not evident penetration; 3. Tumour structures at the serosal surface of the tumour; c. Vascular invasion: 1) none, 2) possible, 3) well established. Total score variation is equal to 8-24. The histologic grading index is then defined as the mean of the values of the eight parameters and ranges from 1 to 3. The index considered by the authors correlates significantly with both presence of tumour at second-look operation and survival.

Traditional grading parameters in the last two decades have been supplemented with several markers of cellular proliferation such as DNA flow cytometry, PCNA-proliferative fraction, Ki-67 expression and assessment of silver-stained nucleolar organizer regions (AgNORs).

In spite of some methodical difficulties and sources of errors, DNA flow cytometry has gained popularity among practical pathologists [5-7]. The method enables assessment of two main parameters – ploidy and the quantity of cells in S phase. The majority of the researchers consider the S–phase cells more important than ploidy since the correlation between grading and ploidy is less significant [8]. It is of interest to note that Haapasalo *et al.* [6] found a close correlation between ploidy and the nuclear perimeter, and also that the surface and nuclear diameter-nondiploid nuclei were larger. From this point of view flow cytometry (in the sense of low ploidy and high ploidy) can be substituted by morphometric parameters, e.g. nuclear perimeter.

PCNA-proliferative fraction for grading of ovarian carcinoma was directly correlated with histological grading. Tumours with a high PCNA expression had a greater frequency of macroscopically-detected residual tumours [9].

The patterns of Ki-67 expression in ovarian epithelial tumours vary significantly. The percentage of Ki-67 staining correlates with DNA index, architectural grade, nucleolar grade, and mitotic count in malignant tumours, with nucleolar grade in benign tumours but with none of these variables in borderline tumours [10]. Other authors also failed to show any correlation between Ki-67 (MIB-1) labeling index and histological grade, though a significant relationship was observed between the Ki-67 index and disease-free survival in serous ovarian tumours [11-14] Only Marx *et al.* consider that measurement of the growth fraction estimated by MIB-1 more closely reflects the degree of tumour differentiation [15].

Assessment of silver stained nucleolar organizer regions (AgNORs) holds promise in providing important prognostic information since association of the mean number of AgNORs per nucleus and the mean percentage of nuclei with more than five AgNORs per nucleus were highly significant with both histological grade and disease stage [16]. Griffiths *et al.* [17] suggest that the AgNOR number may be related to nuclear events other than proliferation and DNA ploidy.

In spite of all modifications and implementations of new methods, application of grading systems has led to a great variety of opinions, which range from a total denial of histological grading [18] to over-estimation of its prognostic significance [19]. However, the majority of authors [20-32] still consider that histological grading is an important prognostic marker, though in need of serious modifications.

There are several major problems of intraobserver and interobserver variability both of methodical and conceptual character as well.

First of all, our parameters lack standardization. We need precise morphometrical methods to measure the rate of cellular polymorphism. Caryometric investigations in a simplified manner have demonstrated that measurement of nuclear diameter can be very useful for this purpose. There are even concrete figures indicating the rate of critical nuclear size – more than 1x1 mm – which is prognostically a bad parameter [31]. Instead of traditional counting of mitotic figures a new method-volume corrected mitotic index (VCM/I) has been proposed [32]. The principal difference with the old method is that the mitotic figures are counted per square millimeter of epithelium in the microscopic field. VCM/I helps to assess the mitotic activity respecti-

vely to the structural specificity of the given tumour. Such innovation is important for grading tumours which contain many extracellular substances such as mucus, blood, etc. For instance the same quantity of mitotic figures assessed by traditional counting methods in the 10 hpf in serous and mucinous carcinomas does not correspond to the same mitotic activity in these tumours – in the mucinous neoplasms vast territories are occupied by cellular foci filled with mucin. The results of application of VCM/I show that this index correlates better with prognosis. One more useful morphometric parameter is the volume percentage of epithelium [22]. Patients with low values of VPE generally have a good prognosis.

Each parameter should be carefully estimated in accordance with the morphogenetic peculiarities of the given tumour. For instance some grading schemes set off tubular structures against papillar ones. However in many cancers tubules are formed on the papillae, so we can speak strictly about tubulo-papillar structures. From this point of view the innovation of Shimizu, *et al.*, which assigns a low score to the tubules different from papillae, cannot always be applied to practice since both types of structures often coexist. Moreover, in clear cell carcinoma that has a bad prognosis, the majority of the structures may be represented by tubules, thus the structure itself is not a predictive prognostic factor.

It is also very questionable whether or not to accept the introduction of several parameters which are quite good for other localizations but not for ovarian cancer. By this we mean the peculiarities of the periphery of the tumour and the penetration of the capsule. Such approaches can be used in the organs with marked vertical anisomorphism of the wall layers, for instance, in cervical or endometrial cancer. In fully developed ovarian cancer one cannot find central and peripheral parts of the tumour since theoretically just the periphery of the ovary is the site of origin of serous cancer and additionally, the growth most probably occurs in a multinodular manner. The same applies to the identification of penetration of the capsule. If rupture of mucinous tumours is easily diagnosed and serves as a very important marker, in serous cancer it is practically impossible to discriminate invasion of the capsule by cancer cells from foci of the initial surface growth of cancer.

The next unresolved problem is the unsatisfactory completeness of the analyzed clinical data. Thorough analysis of the clinical presentation of the disease is very important for proper assessment of the survival rate. One of the major sources of discrepancies when assessing the results of grading is either lack of information on the stage or misinterpretation of the stage. Many patients, even with properly staged disease belonging to the same stage, very often represent the different extents of the process. For instance, out of two patients with serous ovarian cancer stage IIIc – one may have a tumour involving both ovaries and abdominal implants greater than 2 cm in diameter, the other – tumour involving one ovary and positive inguinal nodes. Naturally, they have a different outcome since the amount of residual tumor in the first patient is higher than in the second case and such patients cannot enter the same group of comparison.

We seldom see in the reports information concerning the application of grading, the description of the volume of surgical resection of the primary and metastatic foci or precise localization of the residual masses. The latter, even having a large size may grow freely in the abdominal cavity without compression of the neighbouring structures, while relatively small and solitary nodes can cause obstruction, e.g., of the urinary tract. It is our feeling that more data on additional pathologies, such as cardiovascular diseases, diabetes, endocrine pathology, etc. could facilitate more precise assessment of the grading system. Unfortunately authors practically never provide information on the immediate causes of death of the patients or details of autopsy protocols (if autopsy was performed at all) which would enable us to discard the deaths from complications of the therapy or concomitant diseases. The survival rates of the groups of patients with different grade chemotherapy schedules are not always described. All mentioned details are exclusively paramount since in the majority of the reports the survival rate of the patients with various grades differs slightly only in months and can be influenced by associated pathology as well. The relationship between the stage and grade also has another aspect. We share the opinion of the authors who consider that grading is especially useful for patients with stage 1 disease, most probably because of the complexity of the factors which amass at the late stages of the disease [33].

The last problem, but not the least, is of the conceptual character. We assume that the grading reflects the rate of differentiation of the cancer cells and consequently grading should be applied to those tumours in which the spectrum of cellular differentiation is principally well known, e.g. the mucinous neoplasms among ovarian tumours. The cells of mucinous cancer produce mucus, which serves as a reliable marker of their func-

tional state and thus we have a normal counterpart of enteroid or endocervical epithelium with all stages of maturation for comparison. The great majority of ovarian neoplasms however belong to serous cancer. The spectrum of differentiation of coelomic epithelium which gives rise to serous cancer is absolutely unclear. The neoplasms contain myriads of structures of the cells whose functional peculiarities are obscure. Only a few reports have shed light on the problem by demonstrating that serous carcinomas display such a rare type of differentiation such as choriocarcinomatous [34]. Hence when grading serous cancer we lack the standard prototypes and substitute the notion of cellular maturation by establishing a rate of cellular polymorphism which is not the same. Until we obtain precise knowledge of the functional differentiation of the cells of serous cancer the grading will be formal and hence of little value for clinical implication.

Our final remark concerns the perspectives of grading in ovarian tumors. The analysis of up-to-date literature and our personal experience shows that one of the principal mistakes in research in this field is that we narrow our interests to cellular proliferating activity. The biopsed material reflects the proliferating activity of the cells at given stage and at a given focus. It is well known however that the proliferation rate depends on the milieu of cancer cell growth (e.g., rate of vascularisation can influence intensity of DNA synthesis) and this index is variable. Because of this, the parameters mentioned would not be valid if assessed only in one focus or one biopsy without data about the rate of their expression in dissemination foci. Moreover, recurrent tumours not obligatory retain the same intensity of proliferation as the primary had. From a purely mathematical point of view data obtained for grading lack discrete character and represent the row of semiqantitative parameters. Hence we cannot avoid the most inconvenient feature of the grading systems - presence of intermediate classes called "moderately differentiated " tumours. We agree with the authors who consider that grading differences are noticeable only in grading G1 and G3. G2 (1/3 of all carcinomas) is a collecting "pool" and its prognosis cannot be assessed [8].

On a large scale the capacity to proliferate is a very important feature of cancer cells but evidently is not a unique property, which determines their metastatic potential. Factors which might be responsible for cellular interaction, cellular fusion, chemical composition of the membranes, etc., should enter the field of extensive investigations of the pathologists and related branches of biology.

According to the latest views on cancer development [35] virtually all types of human tumours including their metastatic overgrowths continue to harbor complex mixtures of several cell types that collaborate to create malignant growth (fibroblasts, immune cells, endothelial cells). Thus the malignancy grade of the tumours should be systemic, including the characteristics not only of the isolated cancer cells but their supporting conspirators. Of course, molecular genetic studies, revealing the finest mechanisms of cellular metabolism, remain the high road of modern oncology which integrates all mentioned facets of the problem and promises fast advancement to the elaboration of new diagnostic and prognostic tools. This facet of the problem however, with all the advantages and disadvantages, is of special interest and merits separate discussion.

References

[1] Broders A. C.: "Carcinoma: Grading and practical application". *Arch. Pathol.*, 1926, *2*, 376.
[2] Russel P.: "The pathological assessment of ovarian neoplasms, III: The malignant "epithelial" tumours". *Pathology*, 1979, *11*, 493.
[3] Shimizu Y., Kamoi S., Amada S., Akiyama F., Silverberg S. G.: "Toward the development of a universal grading system for ovarian epithelial carcinoma". *Cancer*, 1998, *82* (5), 893.
[4] Bichel P., Jakobsen A.: "A new histologic grading index in ovarian carcinoma". *Int. J. Gynecol. Pathol.*, 1989, *8*, 147.
[5] Friedlander M. L.,Taylor I. W., Russel P., Musgrove E. A., Hedley D. H., Tattersall M. H.: "Ploidy as a prognostic factor in ovarian cancer". *Int. J. Gynecol. Pathol.*, 1983, *2* (1), 55.
[6] Rodenburg C. J., Cornelisse C. J., Hermans J., Fleuren G. J.: "DNA flow cytometry and morphometry as prognostic indicators in advanced ovarian cancer: a step forward in predicting the clinical outcome". *Gynecol. Oncol.*, 1988; *29* (2): 176.
[7] Haapasalo H., Atkin N. B., Collan Y., Pesonen E., Paljari L.: "Tumour ploidy, morphometry, histological grading and clinical features in ovarian carcinoma: mutual relations". *Anal. Cell. Pathol.*, 1991, *3* (5), 261.
[8] Kuhn W., Feichter G. E., Hanke J., Rummel H. H., Kaufmann M., Schmid H.: "Clinical course of ovarian cancer in relation to morphologic prognostic factors and cell kinetic parameters". *Geburtshilfe Frauenheilkd*, 1987, *47* (7), 446.
[9] Schonborn I., Minguillon C., Reles A., Bartel U., Lichtenegger W.: "Significance of PCNA proliferating fraction for prognosis of ovarian carcinoma". *Geburtshilfe Frauenhilkd*, 1996, *56* (7), 357.
[10] Huettner P. C., Weinberg D. S., Lage J. M.: "Assessment of proliferative activity in ovarian neoplasms by flow and static cytometry". *Am. J. Path.*, 1992, *141* (3), 699.

[11] Darai E., Walker-Combrouze F., Dauge-Geoffroy M. C., Vincent Y., Feldmann G., Madelenat P., Scoazec J. Y:. "Ki-67 expression in 35 borderline ovarian tumors: relation with clinicopathologic parameters and ploidy". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 1998, *76* (2), 175.
[12] Jordan P. A., Kerns B. J., Pence J. C., Kohler H. F., Bast R. C. Jr., Kinney R. B., Berchuck A.: "Determination of proliferation index in advanced ovarian cancer using quantitative image analysis". *Am. J. Clin. Pathol.*, 1993, *99* (6), 736.
[13] Gazzetti G. G., Ciavattini A., Goteri G., DeNictolis M., Stramazzotti D., Lucarini G., Biagini G.: "Ki-67 antigen immunostaining (MIB-1 monoclonal antibody) in serous ovarian tumors: index of proliferative activity with prognostic significance". *Gynecol. Oncol.,* 1995, *56* (2), 169.
[14] Kerns B. J., Jordan P. A., Faerman L. L., Berchuck A., Bast R. C. Jr., Layfield L. J.: "Determination of proliferation index with MIB-1 in advanced ovarian cancer using quantitative image analysis". *Am. J. Clin. Pathol.*, 1994, *101* (2), 192.
[15] Marx D., Meden H., Brune T., Kron M., Korabiowska M., Kuhn W., Schauer A.: "MIB-1 evaluated proliferative activity in ovarian cancer with respect to prognostic significance". *Anticancer. Res.*, 1997, *17* (1B), 775.
[16] Ghazizadeh H., Sasaki G., Araki T., Konishi H., Aihara K.: "Prognostic value of proliferative activity of ovarian carcinoma as revealed by PCNA and AgNOR analyses". *Am. J. Clin. Pathol.*, 1997, Apr., *107* (4), 451.
[17] Griffiths A. P., Cross D., Knigston R. E., Harkin P., Wells M., Quirke P.: "Flow cytometry and AgNORs in benign, borderline, and malignant mucinous and serous tumours of the ovary". *Int. J. Gynecol. Pathol.*, 1993, *12* (4), 307.
[18] Albrecht M., Goepel E., Simon W. E., Trams G.: "Importance of clinically prognostic factors in the treatment of advanced ovarian carcinoma". *Gebirtshilfe Frauenheilkd*, 1985, *45* (7), 482.
[19] Lotze W., Richter P., Sarembe B.: "Intra-arterial chemotherapy in advanced ovarian cancer.1 Study conditions and prognostic factors". Zentralbl. Gynakol., 1987, 109 (7), 443.
[20] Malkasian G. D. Jr., Melton L. J., O'Brien P. C., Green M. H.: "Prognostic significance of histologic classification and grading of epithelial malignancies of the ovary". Am. J. Obstet. Gynecol., 1984, *149*, 274.
[21] Haapasalo H., Collan Y., Seppa A., Gidlund A. L., Atkin N. B., Pesonen E.: "Prognostic value of ovarian carcinoma grading methods - a method comparison study". *Histopathology*, 1990, 16 (1), 1.
[22] Baak J. P., Langley F. A., Talerman A., Delemarre J. F.: "The prognostic variability of ovarian tumor grading by different pathologists". *Gynecol. Oncol.*, 1987, *27* (2), 166.
[23] Frigerio L., Ferrari A., Busci L., Pirondini A., Garsia S., Caldarella M., Pifarotti G.: "Prognosis factors in epithelial tumors of the ovary". *Ann. Ostet. Ginecol. Med. Perinat.*, 1989, *110* (6), 283.
[24] Gargano G., Catino A., Correale M., Lorusso V., Abbate I., Izzi G., Cramarossa A., *et al.*: "Prognostic factors in epithelial ovarian cancer". Eur. J. Gynaecol. Oncol., 1992, *13* (1 Suppl.), 45.
[25] Kaufmann M., Heberling D., Hoeffken H., Markel S.: "Therapy in metastsizing ovarian cancer-survival rate correlated with histological and cytological grading as prognostic factors". *Geburtshilfe Frauenheilkd*, 1983, *43* (1), 15.
[26] Levin L., Lund B., Heintz A. P.: "Advanced ovarian cancer. An overview of multi-variate analyses of prognostic variables with special reference to the role of cytoreductive surgery". Ann. Oncol., 1993, *4* (Suppl. 4), 23.
[27] Sasaki H., Ochiai K., Terashima Y., Mochizuki S., Soda T., Nishimura H., Yakushiji M., Hirabayashi M.: "Prognostic factors of common epithelial ovarian cancer treated by surgery and cisplatin based combination chemotherapy". *Gan. No. Rinsho.*, 1989, *35* (13), 1615.
[28] Shulz B. O., Baker E., Strolz C., Sellin D., Stegner H. E., Krebs D.: "Grading of epithelial ovarian cancer". *Geburtsh. Frauenhilkd*, 1985, *45* (1), 11.
[29] Stegner H. E.: "Morphological prognosis factors in ovarian carcinoma". *Geburtshilfe Frauenheilkd*, 1985, *45* (7), 425.
[30] Vacher-Lavenu M. C., Le Tourneau A., Duvillard P., Godefroy N., Pinel M. C.: "Pathological classification and grading of primary ovarian carcinoma: experience of the ARTAC ovarian study group". *Bull. Cancer*, 1993, *80* (2), 135.
[31] Baak J. P., Wisse-Brekelmans E. C., Langlay F. A., Talerman A., Delemarre J. F.: "Morphometric data to FIGO stage and histological type and grade for prognosis of ovarian tumours". *J. Clin. Pathol.*, 1986, *39* (12), 1340.
[32] Haapsalo H., Pesonen E., Collan Y.: "Volume corrected mitotic index (M/V-INDEX). The standard of mitotic activity in neoplasms". *Path. Res. Pract.*, 1989, *185*, 551.
[33] Powlis W. D., Mauch P., Ehrmann R. L., Rose C. M., Knapp R. C., Bloomer W. D.: "The role of postoperative local or regional irradiation in the treatment of stage 1 ovarian cancer". *Radiology*, 1982, *142* (3), 747.
[34] Oliva E., Andrada E., Pezzica E., Prat J.: "Ovarian carcinoma with choriocarcinomatous differentiation". *Cancer*, 1993, *72* (8), 2441.
[35] Hanahan D., Weinberg A.: "The hallmarks of cancer". *Cell.*, 2000, *100*, 57.

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