

c-erbB-2, p53, and nm23 Proteins as Prognostic Factors in Patients with Epithelial Ovarian Carcinoma

Snježana Tomić, Jadranka Ilić Forko¹, Damir Babić¹, Dinka Šundov, Sendi Kuret, Šimun Anđelinović

Department of Pathology, Split University Hospital Center and School of Medicine, Split; and ¹Department of Gynecological and Prenatal Pathology, Zagreb University Hospital Center and School of Medicine, Zagreb, Croatia

Aim. To demonstrate immunohistochemical expression of p53, c-erbB-2, and nm23 proteins in ovarian cancer and to establish their correlation with such predictive factors as clinical stage, grade, and vascular invasion. The effect of protein overexpression on patients' overall survival was also assessed.

Method. We performed immunohistochemical analysis of formalin-fixed, paraffin-embedded specimens from 80 ovarian carcinomas, using the anti-nm23, p53, and c-erbB-2 monoclonal antibodies. Immunohistochemical results were scored semiquantitatively. All patients were staged according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO) staging system (I-IV). Carcinomas were graded as low- or high-grade, according to the modified grading system recommended by Shimatzu and Silverberg. For univariate analysis, survival time was analyzed by Kaplan-Meier method, and the log-rank test was used to assess the differences between the groups. For multivariate analysis, Cox proportional hazard regression model was used to examine several parameters simultaneously.

Results. Univariate analysis showed that advanced clinical stage ($p < 0.001$); positive staining for nm23 ($p < 0.001$), p53 ($p = 0.021$), and c-erbB-2 ($p = 0.003$) protein; high histological grade ($p < 0.001$); and vascular invasion ($p = 0.006$) were associated with shorter overall survival. Multivariate analysis revealed only clinical stage as an independent prognostic parameter ($p = 0.014$). Multivariate analysis for early-stage disease showed that only the presence of vascular invasion was significantly associated with shorter survival ($p = 0.008$), whereas none of the parameters analyzed for the advanced-stage disease showed independent predictive value for prognosis.

Conclusion. The overexpression of p53, nm23, and c-erbB-2 proteins was associated with other parameters characteristic of aggressive tumors, such as advanced clinical stage, high grade, and/or presence of vascular invasion. However, this overexpression had no independent prognostic value either for overall survival or survival corrected by clinical stages.

Key words: immunohistochemistry; ovarian neoplasms; protein p53; survival rate; tumor markers, biological

In most Western countries, ovarian cancer is the fourth most common cause of death from cancer in women, accounting for 5% of all cancer-related deaths (1). Approximately one-fourth of all gynecologic malignancies are of ovarian origin, and 47% of all gynecologic cancer-related deaths are due to ovarian cancer (1).

The main reason for such a high mortality rate associated with this tumor is the absence of symptoms in the majority of women with early stages of the disease; 70% of women present with symptoms when the disease is already at an advanced stage (2). Extensive intra-abdominal disease is difficult to eradicate surgically and many patients have only a partial response to postoperative chemotherapy. In spite of sig-

nificant advances in surgery and the use of new, more effective chemotherapeutic regimens, the 5-year survival of patients in all stages of the disease is only 30%. It seems that there has been no significant decrease in the incidence or mortality from ovarian cancer since the early 1980s, although some studies reported a decreasing incidence in younger women, attributed to the use of oral contraceptive pill (2).

The prognosis of patients with ovarian tumor depends on surgical factors (stage of the disease and residual disease following laparotomy), response of residual disease to postoperative treatment, and the histopathological characteristics of the tumor (3). Identification of new prognostic factors might be useful in directing the therapy and intensifying the fol-

low-up of a selected group of patients. Finally, the results of research in prognostic factors may provide better understanding of the biological behavior of ovarian tumors.

There is an increasing number of studies on the predictive value of biofunctional markers in determining prognosis and treatment response of patients with epithelial ovarian cancer. Successively, the role of proliferation markers (MIB-1, Ki-67), DNA ploidy, heat shock proteins, CD44, and melanoma-associated antigen 4 (MAGE-4) have been extensively analyzed, as well as functional and structural alterations of oncogenes and tumor suppressor genes (4).

Biomarkers directly involved in drug resistance, such as proteins involved in multidrug resistance and detoxification, have also been tested as indicators of clinical outcome (5). However, due to pronounced biologic, pathologic, and clinical heterogeneity of ovarian tumors, outcome and interpretation of translational studies are often equivocal and no general agreement has been reached about the clinical significance of investigated markers.

Proto-oncogene c-erbB-2 is located on chromosome 17 and encodes a cell-surface glycoprotein similar in structure to the epidermal growth factor receptor (EGFR). EGFR and c-erbB-2 have tyrosine kinase activity, and c-erbB-2 amplification was shown to occur in a number of human tumors (4). Whereas overexpression has been associated with a poor prognosis of patients with cancer arising from other primary sites (6), studies of the ovarian cancer have produced conflicting results. To date, few reports have been published on c-erbB-2 protein overexpression in specimens of ovarian cancer. Published results are discordant and their clinical value is not clearly established. Some studies showed no value of c-erbB-2 protein overexpression as a predictor of a long time survival (7-9), whereas others have suggested that overexpression is a marker of poor prognosis (6,10).

p53 is a tumor suppressor gene located on the short arm of chromosome 17. It suppresses cell growth by controlling entry into the S-phase of the cell cycle. Mutation or deletion of p53 gene is believed to result in uncontrolled cell proliferation. Most p53 gene mutations result in stabilization of the protein. In contrast to relatively short half-life of the wild type p53 protein, increased stability of the mutant forms allows their detection by immunohistochemical techniques. Mutations of p53 gene are the most common genetic abnormalities described in human cancers and have been implicated in the pathogenesis of a variety of human tumors. Studies have demonstrated an association between p53 protein overexpression and poor prognosis in patients with various tumor types, including breast, colorectal, and lung cancer (11). The overexpression of p53 protein has been reported in up to 81% of cases of ovarian cancer (12). The prognostic significance of this overexpression is controversial. Some authors have found an association between p53 overexpression and poor prognosis (13-15); others have failed to confirm these findings (12).

Gene nm23 was initially cloned as a metastasis-suppressor gene and its expression leads to reduction of tumor metastasis. Reduced nm23 expression has been associated with the presence of metastasis or poorer prognosis in patients with breast carcinoma, melanoma, or colorectal carcinoma (16,17). However, high nm23 expression has been reported to correlate with carcinogenesis or progression of pancreatic carcinoma and neuroblastoma (18). Significance of nm23 expression in human cancers may differ depending on the organs in which the tumors develop. Little data are available on nm23 expression in ovarian carcinoma. Most studies found nm23 overexpression associated with more aggressive tumor phenotype (19,20), and only a few indicated that nm23 overexpression might have a favorable prognostic role in ovarian cancer (21).

We studied p53, c-erbB-2, and nm23 overexpression in patients with epithelial ovarian cancer, for whom we had extensive and complete follow-up information. Our aim was to investigate whether p53, nm23, and c-erbB-2 overexpression was associated with recognized prognostic factors in ovarian cancer, such as stage, grade, and vascular invasion. We also analyzed the association of p53, nm23, and c-erbB-2 overexpression with overall survival and outcome in advanced- and early-stage disease.

Patients and Methods

Clinical Data

Histologic samples of 80 ovarian cancers were retrieved from the files of the Department of Pathology, Split University Hospital Center, Split, from 1989 to 1993. All samples had been taken at laparotomy before the initiation of chemotherapy. Atypical proliferating tumors were not included in the study.

Survival time of the 80 patients included in the study was calculated as the interval from the date of diagnosis to the last clinical control or death from the ovarian cancer-related cause, or until December 31, 1999.

The mean age of patients was 58.2 ± 10.78 years (median, 59; range, 34-79). Median follow-up was 21 month (range, 1-112 months).

All patients were staged according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO) staging system IV (22). Carcinomas were graded as either low- (score 3-6) or high-grade (score 7-9) carcinoma, according to the modified grading system recommended by Shimatzu and Silverberg (23).

Immunohistochemical Staining

At the time of surgery, tumors were dissected and fixed for 24 h in neutral buffered formalin. After fixation, blocks were routinely embedded in paraffin wax.

Immunostaining for c-erbB-2, p53, and nm23 proteins was performed with monoclonal antibodies to human p53 (DAKO, Glostrup, Denmark, mouse anti-human, M7991) at a dilution of 1:50, to c-erbB-2 (DAKO, rabbit anti-human, A0096) at a dilution of 1:100, and to nm23 (DAKO, rabbit anti-human, A0485) at a dilution of 1:200. Before staining, antibodies were diluted in tromethamine (TRIS)-NaCl buffer containing 1% bovine albumin.

Four-micrometer sections were first deparaffinized in xylene and rehydrated in descending concentrations of alcohol. The slides were treated in a microwave oven at 750 W and 110 °C, three times for 5 minutes in a citrate buffer. After this step, slides were stained with labeled streptavidin-biotin and then with chromogen diaminobenzidine (DAKO). For counterstaining, Mayer's hematoxylin was used.

For the determination of c-erbB-2 protein overexpression, only the membrane staining intensity was evaluated. Staining of

the entire membrane in more than 10% of the tumor cells was considered positive (24).

Immunoreactivity to nm23 was invariably cytoplasmatic and perinuclear. Moderately and strongly expressed staining results were considered positive (25).

Positive staining for p53 was nuclear. The results of the p53 immunoreactivity were recorded as positive if distinct nuclear staining was seen in at least 10% of tumor cells. Since proliferating normal tissues show a small proportion of cells with p53 immunostaining, a cut-off point for p53 overexpression was 10% positively immunostained nuclei (13).

Statistical Analysis

For univariate analysis, survival time was analyzed by the Kaplan-Meier method, and the log-rank test was used to assess differences among groups. For multivariate analysis, Cox proportional hazard regression model was used to examine all factors found to be predictive of survival in univariate analysis simultaneously. Associations between tested parameters were studied by Spearman rank correlation. Differences were considered statistically significant at $p \leq 0.05$.

Results

As revealed by the frequency distribution of clinical, histopathological, and immunohistochemical data of our patients with ovarian cancer, there was equal number of patients with low- and high-grade cancer (Table 1). Approximately two-thirds were in the advanced stage of the disease.

There was a clear correlation between p53 protein overexpression and vascular invasion ($p = 0.008$), whereas no significant association could be found between p53-positive staining and advanced clinical stage (FIGO stage III or IV) or high grade (Table 2). Positive staining for c-erbB-2 was significantly associated with vascular invasion ($p = 0.050$), high grade

Table 1. Clinical, histopathological, and immunohistochemical data of 80 patients with ovarian cancer

Parameter	No. of patients
Histological grade:	
low (score 3-6)	40
high (score 7-9)	40
Clinical stage:	
early (I and II)	27
advanced (III and IV)	53
Vascular invasion:	
yes	55
no	25
p53:	
+	60
-	20
c-erbB-2:	
+	48
-	32
nm23:	
+	60
-	20

Table 2. Association between overexpression of p53 and clinicopathological variables

Variable	No. of patients	p53 ⁺	p53 ⁻	R*	p
Clinical stage:					
I and II	27	18	9	0.137	0.224
III and IV	53	42	11		
Grade:					
low	40	8	12	0.115	0.308
high	40	32	8		
Vascular invasion:					
yes	55	46	9	-0.296	0.008
no	25	14	11		

*Spearman's rank correlation coefficient.

Table 3. Association between overexpression of c-erbB-2 and clinicopathological variables

Variable	No. of patients	c-erbB-2 ⁺	c-erbB-2 ⁻	R*	p
Clinical stage:					
I and II	27	12	15	0.227	0.043
III and IV	53	36	17		
Grade:					
low	40	28	12	0.255	0.022
high	40	29	11		
Vascular invasion:					
yes	55	37	18	-0.220	0.050
no	25	11	14		

*Spearman's rank correlation coefficient.

Table 4. Association between overexpression of nm23 and clinicopathological variables

Variable	No. of patients	nm23 ⁺	nm23 ⁻	R*	p
Clinical stage:					
I and II	27	14	13	0.382	<0.001
III and IV	53	46	7		
Grade:					
low	40	24	16	0.362	0.002
high	40	36	4		
Vascular invasion:					
yes	55	48	7	-0.420	<0.001
no	25	11	14		

*Spearman's rank correlation coefficient.

($p = 0.022$), and advanced clinical stage of the disease ($p = 0.043$) (Table 3). Positive staining for nm23 was associated with all analyzed parameters: vascular invasion ($p < 0.001$), high grade ($p = 0.002$), and advanced clinical stage ($p < 0.001$) (Table 4).

Univariate analysis showed that advanced clinical stage ($p < 0.001$), high grade ($p < 0.001$), vascular invasion ($p < 0.001$), and positive staining for p53 ($p = 0.021$), nm23 ($p < 0.001$), and c-erbB-2 ($p = 0.003$) protein were all associated with shorter overall survival (Table 5). When all these predictors were included in Cox multivariate analysis (Table 5), clinical stage was the only predictor significantly associated with survival ($p = 0.014$). Other parameters showed no independent value for predicting the prognosis of patients with ovarian cancer.

Using Cox proportional hazard regression analysis, we examined the association between grade, vascular invasion, and expression of p53, c-erbB-2, and nm23 proteins with patient survival in early (FIGO stage I and II) and advanced (III and IV) clinical stage (Table 6). Multivariate analysis of early-stage disease showed that only the presence of vascular invasion was significantly associated with survival ($p = 0.008$), whereas in the advanced stage of disease, none of the other variables showed any independent predictive value for patient prognosis.

Discussion

We used immunohistochemical approach to investigate p53, nm23, and c-erbB-2 protein overexpression in ovarian cancer to assess the frequency of their occurrence, their relationship with clinicopathological parameters and biological aggressiveness of tumor, and their role in tumor progression.

No correlation was found between p53 protein overexpression and clinical stage. Some investigators

Table 5. Univariate and multivariate analysis of effects of investigated parameters on overall survival

Variable	Analysis			
	univariate log rank (p)	HR*	multivariate 95% CI [†]	p
Clinical stage (I + II vs III + IV)	27.86 (<0.001)	1.747	1.122-2.721	0.014
c-erbB-2 (+, -)	9.10 (0.003)	1.258	0.636-2.489	0.509
p53 (+, -)	5.34 (0.021)	0.956	0.425-2.149	0.913
nm23 (+, -)	13.07 (<0.001)	1.760	0.676-4.582	0.247
Histological grade (low, high)	26.50 (<0.001)	1.138	0.587-2.205	0.702
Vascular invasion (no, yes)	39.02 (<0.001)	0.280	0.078-1.004	0.051

*Hazard ratio.

[†]Confidence interval.**Table 6.** Cox proportional hazard regression analysis of patients in early and advanced stage of the disease

Variable	Early stage (I and II)			Advanced stage (III and IV)		
	HR*	95% CI [†]	p	HR	95% CI	p
c-erbB-2 (+, -)	0.396	0.050-3.155	0.382	1.465	0.661-3.249	0.347
p53 (+, -)	1.576	0.142-17.521	0.711	0.843	0.354-2.007	0.700
nm23 (+, -)	0.854	0.106-6.875	0.882	2.329	0.676-8.030	0.181
Histological grade (low, high)	1.307	0.145-11.800	0.811	1.109	0.551-2.229	0.772
Vascular invasion (no, yes)	0.021	0.001-0.372	0.008 [‡]	0.724	0.188-2.785	0.639

*Hazard ratio.

[†]Confidence interval.

found that p53 overexpression was more common in tumors in advanced than in early stage (13,14). In other studies p53 overexpression was not associated with the stage of disease (12,15). In our study, overexpression of p53 gene significantly correlated with vascular invasion, but not with the clinical stage and high histological grade of the tumor. The association between vascular invasion and p53 overexpression in ovarian cancer has not been previously reported, but Goodheart et al (26) described correlation between p53 mutation and microvessel density count in ovarian cancer. Their finding was consistent with the hypothesis that wild-type p53 gene inhibits angiogenesis by inducing the synthesis of angiogenic thrombospondin-1. If p53 gene is mutated, the level of thrombospondin-1 drops, shifting the balance in favor of angiogenic factors, helping the growth and spread of the tumor (11).

Overexpression of nm23 and c-erbB-2 proteins could be an indication of a more aggressive phenotype because it significantly correlated with the advanced clinical stage, vascular invasion, and high grade of tumor. Some authors found correlation between overexpression of c-erbB-2 gene and advanced clinical stage and high histological grade (27,28). In other studies, c-erbB-2 overexpression was not associated either with clinical stage of disease or with histological grade (7,29).

Although univariate analysis in our study showed that all parameters analyzed were associated with shorter survival, multivariate analysis revealed that only clinical stage was significantly associated with shorter survival.

The prognostic value of stage according to the FIGO has been well established (3). The importance of staging with regard to survival also stems from the influence it has on subsequent patient management. Extensive surgical staging in presumed FIGO I and II stage of ovarian cancer showed that 31% were actually in a more advanced stage, mostly stage III (3). These patients are likely to be given inadequate treat-

ment if metastatic spread outside of ovaries is overlooked. Although the FIGO stage provides a fairly accurate assessment of the prognosis, there is a controversy over some aspects of surgical staging procedure, especially regarding the status of retroperitoneal lymph nodes. Opponents to "intensive surgical staging" point out that aggressive operation cannot significantly change the survival of the patients, but it can increase the operation risk. Surgeons who operated on patients included in this study were of the same opinion – adequate para-aortic lymphnode sampling was rarely done. We believe that this fact could account for the significant association of vascular invasion with the survival of the patients in the early, but not in the advanced stages of the diseases.

In our study, univariate analysis showed association of p53, nm23, and c-erbB-2 protein overexpression with shorter overall survival. However, these overexpressed proteins were not proven as independent prognostic indicators by multivariate analysis, either in early or in advanced clinical stage of the disease.

Researchers have been trying to assess the real prognostic significance of p53, nm23, and c-erbB-2 overexpression in ovarian cancer, but results of their studies have been controversial. For instance, some studies found that nm23 overexpression was connected with more aggressive tumor phenotype and shorter survival (19,20), whereas other studies indicated that nm23 overexpression might have a favorable prognostic role in ovarian cancer (21,30).

Wen et al (13), Dong et al (14), and Bossari et al (15) have found an association between p53 overexpression and poor prognosis, whereas others have failed to confirm these findings (12).

Regarding the c-erbB-2 protein overexpression, Medl et al (7), Frutuoso et al (8), and Afify et al (9) showed that c-erbB-2 overexpression could not be used as a predictor of long time survival, whereas Slamon et al (6) and Berchuck et al (10) suggested that overexpression was a marker of poor prognosis.

The results of studies on the influence of c-erbB-2, nm23, and p53 overexpression on survival in early and advanced clinical stages has have not been uniform either. Bossari et al (15) found that p53 protein overexpression was associated with shorter survival of patients in early but not in advanced stage of the disease. Hartman et al (31), on the other hand, concluded that p53 overexpression was associated with shorter survival in advanced but not in early stage of the disease. Meden et al (32) found that c-erbB-2 overexpression had a statistically significant negative prognostic effect in early stage patients, but results of Leeson et al (33), which were similar to our results, indicated that c-erbB-2 overexpression had no prognostic significance in early-stage ovarian carcinoma.

In previous studies of c-erbB-2, nm23, and p53 protein overexpression as a prognostic marker in ovarian carcinoma, various methods were used to detect overexpression, ranging from frozen tissue immunohistochemistry to immunohistochemistry of formalin-fixed paraffin-embedded tissue. However, enzyme and microwave treatment of the tissue during the staining process may greatly affect the staining results, and tissue fixation procedures may also influence immunostaining.

Different scoring methods and subjective interpretation of immunohistochemical analysis may also be reasons for different results obtained by different studies. In ovarian cancer research, further controversy is created by the use of different antibodies, different cut-off level between positive and negative tumors, and inadequate staging and follow-up of the patients.

What role, if any, c-erbB-2, nm23, and p53 genes play in the etiology, pathogenesis, or biologic behavior of ovarian cancer remains unknown. The results of our study support the view that the presence of overexpression is associated with other parameters characteristic of aggressive tumors and that overexpression has no independent value for patient prognosis. Uniformly poor prognosis of patients with advanced-stage ovarian cancer might well obscure subtle variations in prognosis related to the expression of these genes. Although we could not confirm the significance of overexpression of these proteins as prognostic factors in our group of patients with ovarian cancer, they should be considered as potential targets for studies in antibody-directed therapy or gene therapy. Therapeutic strategies targeted at c-erbB-2 are under development, such as the use of monoclonal receptor antibodies to inhibit tyrosine kinase activity. Also, the fact that acquired mutation in the p53 tumor suppressor gene is the most frequent genetic alteration in ovarian cancer makes this gene an ideal molecular target for gene therapy.

References

- Cannistra SA. Cancer of the ovary. *N Engl J Med* 1993; 329:1550-9.
- Gross TP, Schlesselman JJ. The estimated effect of oral contraceptive use on the cumulative risk of epithelial ovarian cancer. *Obstet Gynecol* 1994;83:419-24.
- Baak JP, Chan KK, Stolk JG, Kenemans P. Prognostic factors in borderline and invasive ovarian tumors of the common epithelial type. *Pathol Res Pract* 1987;182: 755-74.
- Geisler JP, Geisler HE. Tumor markers and molecular biological markers in gynecologic malignancies. *Curr Opin Obstet Gynecol* 2001;13:31-9.
- Arts HJ, Van Der Zee AG, de Jong S, de Vries EG. Options for modulation of drug resistance in ovarian cancer. *Int J Gynecol Cancer* 2000;10:47-52.
- Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 1989;244:707-12.
- Medl M, Sevelda P, Czerwenka K, Dobianer K, Hanak H, Hruza C, et al. DNA amplification of HER-2/neu and INT-2 oncogenes in epithelial ovarian cancer. *Gynecol Oncol* 1995;59:321-6.
- Frutuoso C, Silva MR, Amaral N, Martins I, De Oliveira C, De Oliveira HM. Prognosis value of p53, c-erbB-2 and Ki67 proteins in ovarian carcinoma [in Portuguese]. *Acta Med Port* 2001;14:277-83.
- Afify AM, Werness BA, Mark HF. HER-2/neu oncogene amplification in stage I and stage III ovarian papillary serous carcinoma. *Exp Mol Pathol* 1999;66:163-9.
- Berchuck A, Kamel A, Whitaker R, Kerns B, Olt G, Kinney R, et al. Over expression of HER-2/neu is associated with poor survival in advanced epithelial ovarian cancer. *Cancer Res* 1990;50:4087-91.
- Cotran RS. Neoplasia. In: Cotran RS, Kumar V, Collins T, editors. *Robbins pathologic basis of disease*. 6th ed. Philadelphia: W.B. Saunders Company; 1999. p. 276-98.
- Kupryjanczyk J, Bell DA, Yandell DW, Scully RE, Thor AD. p53 expression in ovarian borderline tumors and stage I carcinomas. *Am J Clin Pathol* 1994;102:671-6.
- Wen WH, Reles A, Runnebaum IB, Sullivan-Halley J, Bernstein L, Jones LA, et al. p53 mutations and expression in ovarian cancers: correlation with overall survival. *Int J Gynecol Pathol* 1999;18:29-41.
- Dong Y, Walsh MD, McGuckin MA, Cummings MC, Gabrieli BG, Wright GR, et al. Reduced expression of retinoblastoma gene product (pRb) and high expression of p53 are associated with poor prognosis in ovarian cancer. *Int J Cancer* 1997;74:407-15.
- Bosari S, Viale G, Radaelli U, Bossi P, Bonoldi E, Coggi G. p53 accumulation in ovarian carcinomas and its prognostic implications. *Hum Pathol* 1993;24:1175-9.
- Sawan A, Lascu I, Veron M, Anderson JJ, Wright C, Horne CH, et al. NDP-K/nm23 expression in human breast cancer in relation to relapse, survival and other prognostic factors: an immunohistochemical study. *J Pathol* 1994;172:27-34.
- Royds JA, Cross SS, Silcocks PB, Scholefield JH, Rees RC, Stephenson TJ. Nm23 "anti-metastatic" gene product expression in colorectal carcinoma. *J Pathol* 1994; 172:261-6.
- Leone A, Seeger RC, Hong CM, Hu YY, Arboleda MJ, Brodeur GM, et al. Evidence for nm23 RNA overexpression, DNA amplification and mutation in aggressive childhood neuroblastomas. *Oncogene* 1993;8: 855-65.
- Srivatsa PJ, Cliby WA, Keeney GL, Dodson MK, Suman VJ, Roche PC, et al. Elevated nm23 protein expression is correlated with diminished progression-free survival in patients with epithelial ovarian carcinoma. *Gynecol Oncol* 1996;60:363-72.

- 20 Leary JA, Kerr J, Chenevix-Trench G, Doris CP, Hurst T, Houghton CR, et al. Increased expression of the NME1 gene is associated with metastasis in epithelial ovarian cancer. *Int J Cancer* 1995;64:189-95.
- 21 Ferrandina G, Scambia G, Marone M, Benedetti Panici P, Giannitelli C, Pernisco S, et al. nm23 in ovarian cancer. Correlation with clinicopathological and biochemical parameters. *Ann N Y Acad Sci* 1996;784:509-12.
- 22 Benedet JL, Bender H, Jones H 3rd, Ngan HY, Pecorelli S. FIGO staging classifications and clinical practice guidelines in the management of gynecologic cancers. FIGO Committee on Gynecologic Oncology. *Int J Gynaecol Obstet* 2000;70:209-62.
- 23 Silverberg SG. Histopathologic grading of ovarian carcinoma: a review and proposal. *Int J Gynecol Pathol* 2000;19:7-15.
- 24 Singleton TP, Niehans GA, Gu F, Litz CE, Hagen K, Qiu Q, et al. Detection of c-erbB-2 activation in paraffin embedded tissue by immunohistochemistry. *Hum Pathol* 1992;23:1141-50.
- 25 Schneider J, Romero H, Ruiz R, Centeno MM, Rodriguez-Escudero FJ. nm23 expression in advanced and borderline ovarian carcinoma. *Anticancer Res* 1996;16:1197-202.
- 26 Goodheart MJ, Vasef MA, Sood AK, Davis CS, Buller RE. Ovarian cancer p53 mutation is associated with tumor microvessel density. *Gynecol Oncol* 2002;86:85-90.
- 27 Kacinski BM, Mayer AG, King BL, Carter D, Chambers SK. Neu protein overexpression in benign, borderline, and malignant ovarian neoplasms. *Gynecol Oncol* 1992;44:245-53.
- 28 Wang DP, Konishi I, Koshiyama M, Nanbu Y, Iwai T, Nonogaki H, et al. Immunohistochemical localization of c-erbB-2 protein and epidermal growth factor receptor in normal surface epithelium, surface inclusion cysts, and common epithelial tumours of the ovary. *Virchows Arch A Pathol Anat Histopathol* 1992;421:393-400.
- 29 Felip E, Del Campo JM, Rubio D, Vidal MT, Colomer R, Bermejo B. Overexpression of c-erbB-2 in epithelial ovarian cancer. Prognostic value and relationship with response to chemotherapy. *Cancer* 1995;75:2147-52.
- 30 Kapitanović S, Spaventi R, Vujsić S, Petrović Z, Kurjak A, Pavelić ZP, et al. nm23-H1 gene expression in ovarian tumors – a potential tumor marker. *Anticancer Res* 1995;15:587-90.
- 31 Hartmann LC, Podratz KC, Keeney GL, Kamel NA, Edmonson JH, Grill JP, et al. Prognostic significance of p53 immunostaining in epithelial ovarian cancer. *J Clin Oncol* 1994;12:64-9.
- 32 Meden H, Kuhn W. Overexpression of the oncogene c-erbB-2 (HER2/neu) in ovarian cancer: a new prognostic factor. *Eur J Obstet Gynecol Reprod Biol* 1997;71:173-9.
- 33 Leeson SC, Morphopoulos G, Buckley CH, Hale RJ. c-erbB-2 oncogene expression in Stage I epithelial ovarian cancer. *Br J Obstet Gynaecol* 1995;102:65-7.

Received: March 11, 2003

Accepted: July 1, 2003

Correspondence to:

Snježana Tomić
Department of Pathology
Split University Hospital Center
Spinčićeva 1
21000 Split, Croatia
snjezana.tomic@st.hinet.hr