Prognostic value of creatine kinase BB isoenzyme in epithelial ovarian carcinoma

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ZARGHAMI N, KATSAROS D, YU H, DIAMANDIS EP. Prognostic value of creatine kinase BB isoenzyme in epithelial ovarian carcinoma. The Canadian Journal of Oncology. 1995;5(3):401-407.

Creatine kinase BB isoenzyme (CK-BB) is overexpressed in many tumor tissues, including ovarian cancer. Using a highly sensitive and specific immunofluorometric method, CK-BB levels in 89 primary ovarian cancer cytosolic extracts have been measured and the associations between CK-BB and clinicopathological features of ovarian cancer have been studied. It was found that CK-BB levels are higher in endometrioid cell carcinomas. No clear association was established between CK-BB levels and patient age, menopausal status, clinical stage, histological grade or size of residual tumor. CK-BB was not associated significantly with either disease-free or overall survival of the patients. Based on these data, it was concluded that there is no prognostic value of CK-BB in ovarian cancer. Drugs that target CK-dependent energy metabolism of tumor cells may not be selective in ovarian cancer therapy.

Key Words: creatine kinase, creatine kinase isoenzymes, ovarian cancer, prognostic factors

Introduction

Creatine kinase (CK, E.C.2.7.3.2) is an important enzyme involved in cellular energy homeostasis. CK catalyzes the phosphorylation and dephosphorylation between creatine and ATP and controls the concentration balance between ATP and ADP. Three major isoenzymes of CK exist in various tissues, each being a dimer of two subunits—the B (brain) and M (muscle) subunits. CK-BB is mainly present in the brain, lung, intestine, bladder, uterus, breast, prostate and placenta. The other two isoenzymes (CK-MM and CK-MB) predominate in the skeletal and cardiac muscles.[1,2]

Cells with high cellular activity, such as brain or skeletal muscle cells, tend to have high levels of total CK due to the large demand for energy expenditure. High levels of CK (or one of its isoenzymes) have also been observed in some primary cancerous tissues,[3-5] metastatic lesions[6] and sera from patients with various cancers.[7-12] Interruption of the energy supply regulated by CK could slow down the growth of cancer cells. Recently, it has been demonstrated in tissue culture studies that, by replacing the normal CK substrate creatine with cyclocreatine, it is possible to significantly suppress the growth or even kill tumor cells.[13-15] Cyclocreatine is an analogue of creatine, and both can serve as substrates for CK. However, compared to creatine (the dephosphorylation of cyclocreatine by CK), transfering energy back to ADP is much more difficult. This results in the block of energy transfer. Without sufficient energy supply, tumor cells stop growing and gradually die; therefore, this agent may have therapeutic potential.

The brain isoenzyme of CK is associated with cancer. It has been found in animal experiments that estrogen could increase the transcription of CK-BB in

the rat uterus.[16,17] As estrogen is suspected to play a pivotal role in the development and progression of several human cancers, potential clinical implications of CK-BB were searched. Elevated serum levels of CK-BB have been seen frequently in patients with breast cancer.[8-10] Cell culture studies have confirmed the capability of estrogen to induce the production of CK-BB in breast cancer cell lines[18]. The transcription of CK-BB is also up-regulated by a protein from adenovirus[19] and this protein could promote malignant transformation, suggesting that increasing the capacity of cells for energy supply may be linked to the oncogenic process. Moreover, a recent study found that the protein encoded by the wild-type p53 tumor suppressor gene from a mouse could inhibit the transcription of CK-BB gene in a rat.[20] The p53 gene is frequently mutated in many types of malignancies. This finding indicates that in cancer, the regulation of energy supply related to CK-BB may be set at a higher point, to meet the need for large energy expenditure during the vicious growth of cancer cells.

Our previous study (Zarghami, Giai, Yu, Roagna, Ponzone, Katsaros, Sismondi and Diamandis; unpublished data, 1995) found that breast cancer patients with high tumor levels of CK-BB tended to have a higher risk for death compared to those with low CK-BB. Although we did not suggest that CK-BB is an independent prognostic indicator in breast cancer, the finding may have utility in the future for selecting patients suitable for a treatment that targets the interruption of energy supply to the cancer cells.

The ovary is another endocrine-related organ in women. Increased CK-BB production by estrogen has been observed in rats[21]. In addition, p53 gene mutations have been detected frequently in ovarian cancer[22]. To our knowledge, no study has been reported describing the cytosolic concentration of CK-BB and its association with clinical features of ovarian cancer. In this study, we have examined some of these associations and their potential for clinical applications.

Materials and methods

Tumor specimens from 90 consecutive patients with histologically confirmed primary epithelial ovarian cancer were collected during surgery at the Department of Gynecologic Oncology of the University of Turin, Italy from 1989 to 1993. The ages of the patients were between 20 to 78 years (median age was 54). The patients were followed clinically after surgery; the follow-up time was between 1.3 and 55.2 months, with a median of 22.2 months. Clinical staging was performed for each patient based on criteria established by FIGO.[23] Each tumor was also histologically graded and typed according to the World Health Organization recommendations.[24] Detailed clinical information on these patients was described elsewhere.[25]

Tumor specimens were snap-frozen in liquid nitrogen immediately after surgical resection and were stored at -80°C until cytosol extraction. Approximately 0.2 g of tumor tissue was pulverized manually at -80°C to a fine powder and the cells were lysed for 30 minutes on ice with 2 mL of lysis buffer (50 mmol/L Tris buffer, pH 8.0, containing 150 mmol sodium chloride, 5 mmol ethylenediaminetetraacetic acid (EDTA), 10 g Nonidet NP-40 surfactant and 1 mmol phenylmethylsulfonyl fluoride per liter). Centrifugation of the lysate was performed afterwards at 15 000 g at 4°C for 30 minutes and the supernatant was used for measuring CK-BB and total protein content concentration.

CK-BB concentration in the ovarian cancer cytosols was measured in duplicate with a time-resolved immunofluorometric procedure previously described elsewhere.[26] The assay has a detection limit of 0.002 ng/mL; a within-run precision (coefficient of variation) of 4-9% and a between-run precision of 6-12%. No cross-reaction with CK-MM and 3% cross-reaction with CK-MB were detected for this assay. Protein concentration in the cytosols was measured with a commercial kit (Pierce Chemical Co., Rockford, IL 61105). The levels of p53 were measured as described previously.[25]

For statistical analysis, CK-BB values were categorized into two groups based on the median value, high CK-BB ($\geq$ median) and low CK-BB ($<$ median). Associations between CK-BB status and other clinicopathological features were examined using the contingency table and chi-square test. The features analyzed include: age at diagnosis ($<$ 40, 40-49, 50-59 and 60+), menopausal status (pre and post), clinical stage (I and II versus III and IV), histological grade (1, 2 and 3), residual tumor size (0, $\leq$ 5 cm and $\geq$ 5 cm), histological type (endometrioid, serous and others) and p53 status (positive versus negative).[25] CK-BB levels in relation to disease-free and overall patient survival were analyzed using the Cox proportional hazards regression model at both univariate and multivariate levels.[27] Kaplan-Meier survival curves[28] were also constructed and the logrank test[29] was used to examine the significance of the differences. Computer software packages called SAS (SAS Institute Inc., Cary, NC) and EGRET (Statistics and Epidemiology Research Corporation, Seattle, WA) were used for the data analysis.

Results

Of the 90 ovarian cancer patients, 89 were assessed for CK-BB levels in their tumor cytosols. CK-BB

Table 1:
Associations between CK-BB status and clinicopathologic variables of ovarian cancer

Variable	CK-BB Low[1]	CK-BB High	p value
Age (yr)			
< 40	6 (13.6)	3 (6.7)	
40-49	10 (22.7)	11 (24.4)	
50-59	17 (38.6)	16 (35.6)	
60+	11 (25.0)	15 (33.3)	0.64
Menopause[2]			
Pre	19 (43.2)	16 (36.4)	
Post	25 (56.8)	28 (63.6)	0.51
Stage			
I-II	13 (29.6)	13 (28.9)	
III-IV	31 (70.5)	32 (71.1)	0.94
Grade			
1	11 (25.0)	4 (8.9)	
2	8 (18.2)	17 (37.8)	
3	25 (56.8)	24 (53.3)	0.038
Residual tumor (cm)			
0	17 (39.5)	16 (35.6)	
≤ 5	10 (23.3)	20 (44.4)	
> 5	16 (37.2)	9 (20.0)	0.071
Histotype			
Endometrioid	5 (11.4)	15 (33.3)	
Serous	16 (36.4)	20 (44.4)	
Others	23 (52.3)	10 (22.2)	0.005
Death			
No	28 (63.6)	34 (75.6)	
Yes	16 (36.4)	11 (24.4)	0.22
Relapse			
No	24 (54.6)	30 (66.7)	
Yes	20 (45.5)	15 (33.3)	0.24
p53			
negative[3]	28 (63.6)	22 (48.9)	
positive	16 (36.4)	23 (51.1)	0.16

1. CK-BB Low is defined as < 189 ng of CK-BB per milligram of total protein (median)
2. Information not available for one patient
3. p53 negative < 3 U/g as described elsewhere (25)

concentrations in the cytosols were widely distributed in these patients, ranging from 1 to 1932 ng/mg of total protein. The concentration values at 25%, 50% and 75% were 56, 189 and 461 ng/mg, respectively. Table 1 presents the results of CK-BB status in association with clinical and pathological variables. The

Table 2.
Associations between CK-BB status and relapse-free or overall survival of ovarian cancer patients

CK-BB Status	RR[1](95% CI[2]) for relapse	p value	RR[1](95%CI[2]) for death	p value
Univariate analysis[3]				
Low CK-BB[4]	1.00		1.00	
High CK-BB	0.75 (0.39-1.52)	0.39	0.73 (0.34-1.57)	0.41
Multivariate analysis[5]				
Low CK-BB	1.00[6]		1.00[7]	
High CK-BB	0.52 (0.23-1.16)	0.11	0.81 (0.32-2.04)	0.65

1. Relative risk
2. Confidence interval
3. 89 patients available for univariate survival analysis
4. For cutoff values see Table 1
5. 87 patients available for multivariate survival analysis
6. Adjusted for menopause, clinical stage, histologic grade, residual tumor, histologic type and p53
7. Adjusted for menopause, histologic grade, residual tumor, histologic type and p53

age and menopausal status distributions were similar between the high CK-BB and low CK-BB categories.

The distributions of clinical stage were virtually identical between the two CK-BB groups. With regard to histological grade, the difference tended to be significant between grade 1 and grade 2 cancers. Twenty-five percent of grade 1 tumors had low CK-BB versus only 9% of tumors with high CK-BB. For moderately differentiated cancers (i.e. grade 2), 18% had low CK-BB versus 38% with high CK-BB. However, a further trend for increasing CK-BB levels in poorly differentiated tumors was not observed. For grade 3 tumors, the difference disappears (57% vs 53% of tumors between the low and high CK-BB groups, respectively). The statistical test for the overall differences in histological grade between the two CK-BB groups was of borderline significance (p=0.038, Table 1) but no trend was observed.

The relationship between CK-BB levels and the size of residual tumors seems to be similar to the relationship between CK-BB and histological grade. Compared to patients without residual tumor, those with residual tumor less than 5 cm tended to have higher CK-BB levels. However, this trend was not sustained in the group of patients with residual tumor larger than 5 cm. Therefore, overall there was no trend indicating that CK-BB levels increase with the size of residual tumor.

In this study, there were 20 (22.5%) patients with endometrioid cell carcinoma, 36 (40.5%) patients with serous cell carcinoma and 33 (37.1%) patients with other carcinomas, including seven clear cell, eight mucinous, 10 undifferentiated and eight unclassified. Significant differences seemed to be present in patients with different histological types of ovarian cancer. Most patients with endometrioid tumors were classified in the high CK-BB category, whereas the majority of patients with other tumors were in the low CK-BB category. Patients with serous cell cancer were more evenly distributed between the two CK-BB groups.

A statistically significant association was not established between p53 and CK-BB status, but a tendency existed indicating that more p53 positive tumors were overexpressing CK-BB. Among the 39 p53-positive tumors, 16 were in the low CK-BB category and 23 were in the high CK-BB category. Among the 50 p53-negative tumors, 28 were in the low CK-BB category and 22 were in the high CK-BB category.

The associations between CK-BB status and patient survival are presented in Table 1 and in more detail in Table 2. The risks for either relapse or death were not significantly different between patients with high or low CK-BB cancer. The results were not altered after controlling for clinical and pathological variables, including menopausal status (or age), clinical stage, histological grade, histological type, residual tumor status and p53 protein status. Figures 1 and 2 show the Kaplan-Meier survival curves for overall and relapse-free survival between the two CK-BB groups, respectively. Similar to the finding from the

Figure 1: Kaplan-Meier overall survival curves of ovarian cancer patients
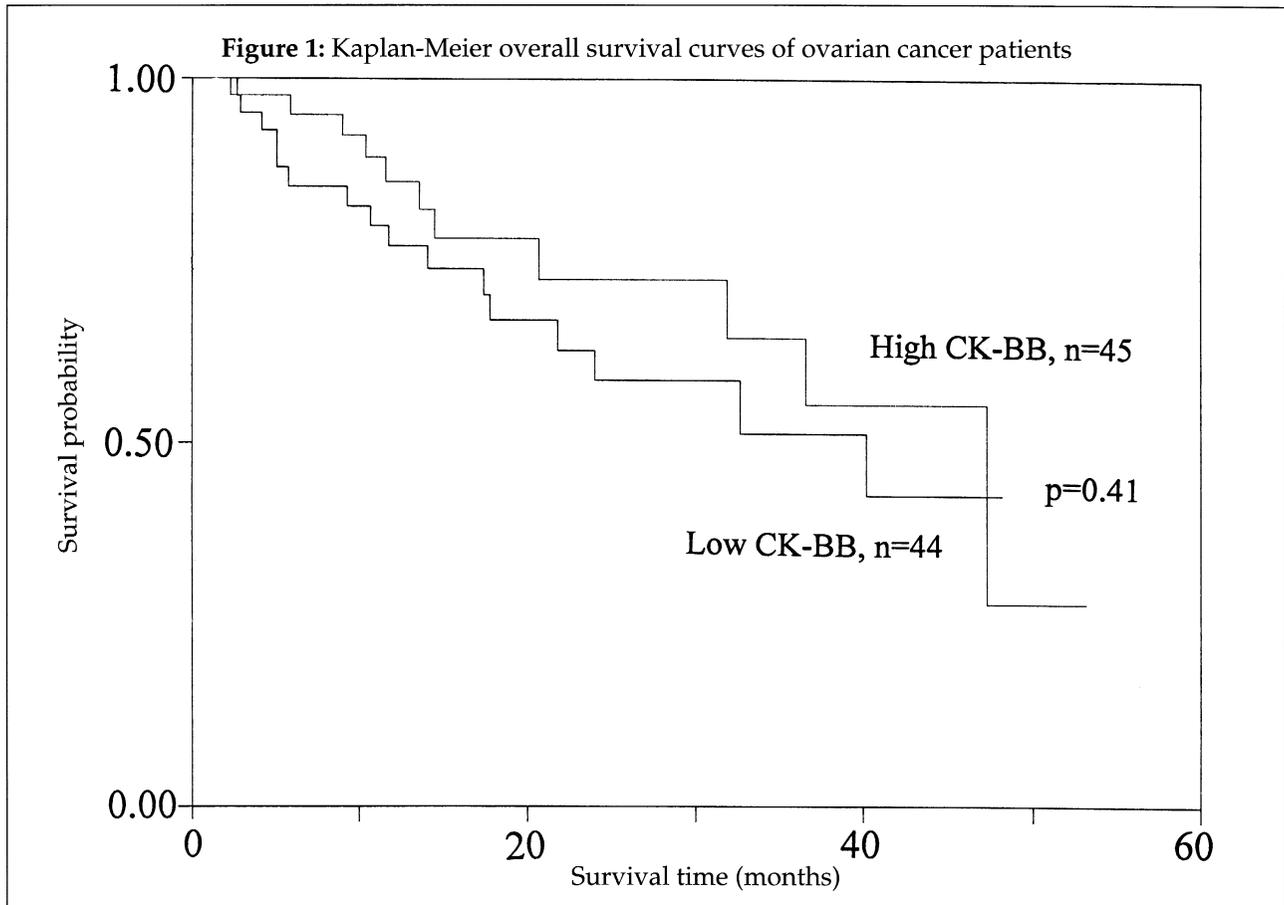
Cox regression models, no significant difference was seen in both relapse-free and overall survivals.

Risks for both overall and relapse-free survivals were also compared between the two CK-BB groups in subsets of patients who were classified based on their histological type, residual tumor status, p53 status and histological grade. No significant difference was noted in any of these analyses (data not shown).

Discussion

Recently evidence emerged suggesting that cellular energy homeostasis associated with CK might be another crucial element involved in cancer development and progression.[19,20] Interruption of the CK metabolic pathway has been suggested to have clinical implications in cancer treatment.[13-15] Previously, we examined CK-BB levels in tumor cytosols of patients with primary breast cancer and found that patients with high levels of CK-BB tended to have shorter overall survival time than those with low levels of CK-BB (in Zarghami, Giai, Yu, Roagna, Ponzone, Katsaros, Sismondi and Diamandis; unpublished data, 1995). Although it needs further confirmation, this observation may indicate a potential future clinical utility. If cyclocreatine treatment is proven to be effective, measuring CK-BB levels in

tumor tissues may help to optimize the selection of patients who might respond better to the treatment.

Little information is available on CK concentrations and its association with clinicopathological features of other cancers. To our knowledge, there has been no study reported so far examining CK-BB levels in tumor cytosols of ovarian cancer and their relationship with various clinical and pathological features of this cancer. We used a sensitive immunoassay to measure CK-BB concentration in the tumor cytosols of ovarian carcinoma and found that CK-BB concentrations varied widely in the ovarian cancer cytosols. After grouping patients into two groups based on the median CK-BB value in these patients, we found that most of the clinicopathological variables were not associated with CK-BB levels. Neither relapse-free survival nor overall survival was shown to be affected by the CK-BB status in ovarian cancer.

Some significant differences in CK-BB status were seen among different histological grades or sizes of residual tumor, but the relationship was difficult to interpret since we have seen neither an increasing nor decreasing trend of the association across all the categories of these variables.

Endometrioid ovarian carcinoma tended to have high levels of CK-BB compared to serous cell or other types. It is not known whether this difference is due

Figure 2: Kaplan-Meier relapse-free survival curves of ovarian cancer patients
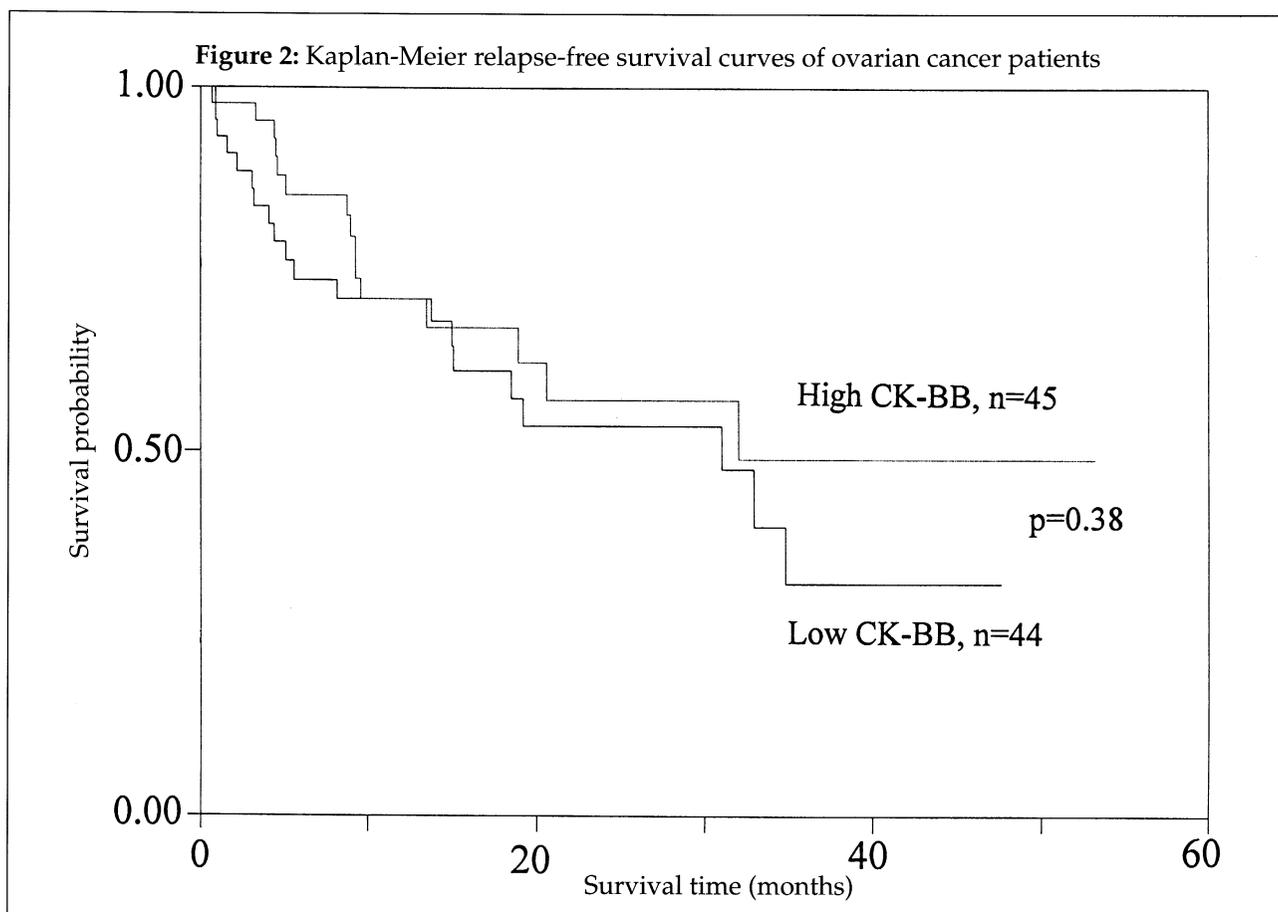
to the cell type per se (i.e., there may be a different energy metabolic pathway in each cell type) or to some other unknown reasons. This observation suggests that the endometrioid type of ovarian cancer may be a candidate for energy interruption treatment if such treatment becomes routinely available.

Another interesting observation in this study is the relationship between CK-BB levels and mutant p53 protein. A recent study[20] has demonstrated that wild type p53 protein could inhibit the transcription of the CK-BB gene. Assuming loss of function of mutant p53 protein in the down-regulation of CK-BB, one may expect increased levels of CK-BB in cancers with p53 gene mutations. Such a tendency was observed in our study but it was not statistically significant. Restraining cellular energy supply could be another important function of the p53 tumor suppressor gene product, which is known to cause cell cycle arrest.

CK-BB concentrations ranged from 5 to 2857 ng/mg in breast cancer cytosols (in Zarghami, Giai, Yu, Roagna, Ponzone, Katsaros, Sismondi and Diamandis; unpublished data, 1995) and from 1 to 1932 ng/mg in ovarian cancer cytosols. Different overall survival in association with CK-BB levels was suggested in breast cancer patients, but not in ovarian cancer patients. The discrepancy of a possible role of CK-BB in the two cancers probably indicates different meta-

bolic pathways for energy expenditure between the two organs. Estrogen has been shown to induce the production of CK-BB[16,17] and the breast is an estrogen-dependent organ in terms of its development and differentiation. The ovary is an estrogen-producing—but not significantly estrogen-dependent—organ compared to the breast. Therefore, CK-BB may not play an important role in the energy homeostasis of the ovarian cells, which may further suggest that the effect of the new treatment that targets the CK metabolic pathway could be organ- or tissue-dependent or -specific.

It is not known whether measuring only CK-BB concentration is adequate for evaluating the overall CK activity in the ovary, since little information is available on the distribution of CK isoenzymes in this organ. In addition, there is a variety of cell types in the ovary and it may be possible that the energy metabolic pathways are different in various cell types. If CK-BB was not the major form of CK in the ovary, we would miss some significant relationships between total CK and ovarian cancer. However, this possibility seems unlikely based on the information available. Tsung measured total CK and the composition of its three isoenzymes in 10 ovarian cancer patients and found CK-BB as the major contributor to the total CK in eight out of the 10 patients[3]. The other two pa-

tients who had CK-MM as the major isoenzyme in the tumor tissues had a diagnosis of lymphoma and granulosa-cell tumor, both of which were not included in our study.

In summary, CK-BB concentrations varied widely in the tumor cytosols of primary ovarian cancer. CK-BB levels tended to be higher in endometrioid cell carcinoma than in other cell types. No clear association was observed between CK-BB concentration and patient's age, menopausal status, clinical stage, histological grade or size of residual tumor. The overall or relapse-free survival of patients with ovarian cancer was not affected by levels of CK-BB. Based on these results, it seems that measuring CK-BB levels in ovarian cancer may not have any clinical implication and that drugs targeting CK-dependant metabolism of tumor cells may not be selective in ovarian cancer therapy. □

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