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Clinical and Prognostic Value of Disseminated Tumor Cells in the Bone Marrow of Patients with Prostate Cancer

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ABSTRACT

Prostate cancer (PC) is one of the most urgent problems of modern clinical urology and oncology, as evidenced by high rates of morbidity and mortality in Ukraine and worldwide. That's why, the search for new predictive and prognostic markers that could be useful for monitoring PC, remains an actual problem. Particularly large interest in disseminated tumor cells (DTC) is concentrated in the context of the clinical course of the PC. To date, there is no unambiguous opinion on the relation between the presence of DTC and the results of treatment of patients with PC. The aim of the work was to investigate the clinical and prognostic value of the DTC in the bone marrow (BM) of patients with PC. For the study, 135 patients with PC of stages I-IV were selected, with an average age of 65.2 ± 9.8 years. Immunocytochemical determination of the DTC in the BM was performed on cytospin preparations of cells isolated from BM samples using monoclonal antibodies against human pancytokeratin (clone AE1/AE3, Dako, Germany). The statistical analysis of the data was carried out using the IBM Statistical 21.0 program. The analysis of the obtained data on the immunocytochemical study of human pancytokeratin expression revealed the presence of DTC in BM in 45.1% of cases. We established the existence of a statistically significant correlation between the presence of DTC in the BM and the clinical and pathological characteristics of patients with PC, such as the spread of the tumor process, the size of the tumor, the presence of metastases in regional lymph nodes, grading by Gleason score, and PSA level in blood serum. We have found that radical surgical treatment of patients with PC can counter-balance the negative effect of this DTC marker in the BM on the course of the disease, namely on the survival of patients and the progression of the tumor process. So, the presence of DTC in the BM of patients with PC could be considered as a marker for an unfavorable prognosis of the disease course.

INTRODUCTION

In practical oncology, many clinical observations have been described, in which, on the background of long-term remission of the disease, the presence of remote metastases in patients with solid tumors was diagnosed [1-4]. This phenomenon is considered to be a period of clinical tumor dormancy and is often observed in cancer patients. In many clinical observations and experimental works, high levels of disseminated (DTC) and circulating tumor cells have been observed in patients with malignant neoplasms, but only a small part of them progresses to form visible metastases [5-7].

Particular interest in the DTC exists in the context of the clinical course of the PC. Exactly for this cancer, the so-called "cancerous dormancy" is typical - a period between the removal of the primary tumor and the relapse of the disease. According to the autopsy data of the University of Washington [8], approximately 90% of patients with PC with advanced disease have metastases in the bones. The DTC can exist separately or in the form of clusters in the bone marrow (BM). According to the literature, DTC in BM are present in 13-72% of patients before radical prostatectomy, and in 20-57% of patients with PC with a recurrence-free period of more than 5 years [9, 10]. The wide range of discrepancies in the detection of DTC can be partly related to the different detection methods used in different laboratories [9-11].

To date, there is no unambiguous opinion on the relation between the presence of DTC and the results of treatment of patients with PC. In the studies of Weckermann et al. and Lilleby et al. [10, 12]it is reported that the detection of the DTC prior to the therapy correlates with relapse of the disease. In contrast, Hung-Ming and co-authors did not find such dependence [13].

For the majority of patients with PC, in which the DTC have been detected after the initial treatment and in the absence of other signs of disease progression, no additional treatment is used before relapse [9, 10].

Taking into account the data on the close relationship between the DTC and bone marrow tissue [14, 15], one may consider rational a therapy targeted on bone microenvironment for eradicating or maintaining a dormancy state. In addition, researchers argue that zoledronic acid in adjuvant mode may have a positive effect on survival rates due to its effect on the DTC [16]. It remains unclear whether zoledronic acid affects the DTC in patients with PC.

So, the search for new predictive and prognostic markers that could be useful for monitoring PC remains an actual issue.

MATERIALS AND METHODS

The study presents the results of a comprehensive analysis of clinical data and morphological features of tumors of 135 patients with PC of stages I-IV, which were treated at the Kyiv City Cancer Hospital and SI "Institute of Urology of the Academy of Medical Sciences of Ukraine" during 2012-2016.

Clinical diagnosis was established on the basis of determination of general prostate specific antigen (PSA) in blood serum, digital rectal prostate examination, CT scan, transrectal ultrasound examination, ultrasonography, osteoscintigraphy, radiography of the chest cavity. Verification of the diagnosis was conducted according to the transrectal multifocal biopsy of the prostate, as well as the tumor biopsy performed by transurethral resection.

The stage of the disease and the extent of the tumor process were determined by the international TNM system (7th edition, 2009). For research work, 135 patients with PC of stage T1-4N0-1 M0-1 were selected. The study involved patients aged 51-75 years (mean age 65.2 ± 9.8 years). The morphological verification of the diagnosis was provided by studying the multifocal biopsy of the prostate gland by conducting a transurethral resection of the prostate before treatment, as well as studying the tumor of the prostate after radical surgical treatment.

By Gleason score, the patients were distributed as follows: variant 3 + 3 = 6 wasin 55 (40.7%) patients; 3 + 4 = 7 in 32 (23.7%) patients; 4 + 3 = 7 in 25 (18.5%); 4 + 4 = 8 in 8 (5.9%) patients, 4 + 5 = 9 in 8 (5.9%) patients; 5 + 4 = 9 in 7 (5.2%) patients.

All patients were monitored for 36 months from the start of diagnosis and treatment.

According to the initial treatment, all the patients under study were divided into 2 groups. The first group - 102 patients who had undergone surgical treatment in the volume of radical prostatectomy with subsequent adjuvant hormone therapy and radiotherapy in the postoperative period. The second group of patients included 33 patients who had conservative treatment in connection with the refusal of the operation and concomitant pathology, which prevented surgical intervention.

In 28 (27.4%) patients from the first group and 8 (24.2%) patients from the second group bilateral operation of orchiectomy was performed. Postoperative radiation therapy was performed in 21 patients from the 1st group, on the prostate tumor – in 21 patients from the 2nd group, palliative radiotherapy in combination with zoledronic acid in the case of progression of the disease due to metastatic bone lesions - in 5 patients from the first group and 13 patients from the second group.

In order to determine the presence of DTC in the BM, all patients before the start of treatment were given sternal puncture in the region of the lower third of the sternum. Mononuclears were isolated from the BM samples in a gradient of density of ficoll-verografin (PAA, Austria), which were used to prepare a series of cytospin preparations for immunocytochemical study. All specimens from patients with PC were received prior to treatment and surgical intervention.

Immunocytochemical determination of the DTC in the BM was performed on cytospin preparations of cells isolated from BM samples using monoclonal antibodies against human pancytokeratin (clone AE1/AE3, Dako, Germany). For visualization of the reaction, the kit Envision+ and 3,3-diaminobenzidine (DakoCytomation, Denmark) were used. After the immunocytochemical reaction, the preparations were stained with a solution of hematoxylin-eosin (15-30 seconds) and placed in the Faramount Aqueous Mounting Medium (ThermoScientific, USA). The presence/absence of DTC in the BM samples was evaluated by the results of an analysis of 3 cytospin preparations for each patient with PC.

The statistical analysis of the data was carried out using the IBM Statistical 21.0 program.

RESULTS

The analysis of the obtained data on the immunocytochemical study of human pancytokeratin expression revealed the presence of DTC in BM in 45.1% of cases. The next stage of our work was the study of the relationship between the presence of DTC in BM of patients with PC and their clinical-pathological characteristics such as disease stage, grading by Gleason score, surgical edge status and PSA level before treatment.

We established the existence of a statistically significant correlation between the degree of spread of the tumor process and the frequency of the presence of DTC in the BM (Spearman's correlation coefficient of 0.313, $p = 0.001$). The proportion of patients with the

presence of DTC in the BM was the highest at III and IV stages of the disease (32 patients, 59.0%), and the smallest proportion - at stage I (3 patients, 4.9%) (Table 1). The proportion of patients with the second stage of the spread of the tumor process among patients in this subgroup was 36.1% (22 patients). Among patients without DTC in the BM, the proportion of patients with stage II was 60.8%, with stage I - 18.9% (14 patients) and III and IV stages - 20.3% (15 patients), which was by 38.7% less than in the case of presence of DTC in the BM.

Table 1 The presence of DTC in BM of patients with PC depending on the stage of the disease

Disease stage	DTC in BM (-) (n=74)		DTC in BM (+) (n=61)	
	n	%	n	%
I	14	18.9	3	4.9
II	45	60.8	22	36.1
III	12	16.2	16	26.2
IV	3	4.1	20	32.8

Note: * - the difference between the groups is statistically significant, p = 0.002

A similar pattern was observed in the assessment of the stages by the TNM classification (2009, 7th edition). In the group of patients with the spread of the tumor process, which corresponded to the T3 category, the number of patients with DTC in the BM (+) was 23 patients (37.7%), and for the T4 category, the number of patients with DTC in the BM (+) - 5 (8.2%). In category T2 such a pattern in the predominance of the proportion of patients with DTC in the BM (+) was not found. The number of patients with T2 with DTC in the BM was 33 (54.1%) versus 56 patients (75.7%) without DTC in the BM. The validity of this pattern was checked by the nonparametric Pearson criterion and the value of p = 0.026 was obtained, which indicated the statistical significance of the revealed relationship between the presence of the DTC in the BM and the category T (Table 2).

Table 2 The presence of DTC in the BM in patients with PC depending on the category T (TNM classification, 2009, 7th edition)

T	DTC in BM (-) (n=74)		DTC in BM (+) (n=61)	
	n	%	n	%
2	56	75.7*	33	54.1
3	16	21.6	23	37.7
4	2	2.7	5	8.2*

Note: * - the difference between the groups is statistically significant, p = 0.026

The existence of a direct correlation between the degree of extent of the tumor process outside the primary focus (Table 3) and the presence of DTC in the BM of patients with PC has been established. In 16 (26.2%) of patients with metastases in regional lymph nodes, the DTC in the BM were revealed. Among patients with DTC in the BM (-), this proportion was only 6.8% (5 patients).

Table 3 The presence of DTC in the BM in patients with PC depending on the category N (TNM classification, 2009, 7th edition)

N	DTC in BM (-) (n=74)		DTC in BM (+) (n=61)	
	n	%	n	%
0	69	93.2*	45	73.8
1	5	6.8	16	26.2*

Note: * - the difference between the groups is statistically significant, p = 0.002

Interestingly, the similar relationship between the presence of DTC in the BM and the index characterizing the aggressiveness of the disease – grading by Gleason score (Table 4) has been observed. Thus, in grading 3 + 6 = 6 and 3 + 4 = 7, patients without DTC in the BM were significantly predominant, and vice versa, in the overwhelming majority, the DTC in the BM were observed in gradings by Gleason score 4 + 4 = 8.4 + 5 = 9, 5 + 4 = 9 (statistically significant difference, p = 0.006). A similar pattern gives grounds to consider the presence of DTC in the BM as a significant indicator, which, together with well-known and recognized markers, characterizes an unfavorable prognosis of the disease.

Table 4 The presence of DTC in the BM in patients with PC depending on the grading by Gleason score

Grading by Gleason score	DTC in BM (-) (n=74)		DTC in BM (+) (n=61)	
	n	%	n	%
3+3=6	38	51.4*	17	27.9
3+4=7	18	24.3	14	23.0
4+3=7	12	16.2	13	21.3
4+4=8	4	5.4	4	6.6*
4+5=9	2	2.7	6	9.8*
5+4=9	0	0.0	7	11.5*

Note: * - the difference between the groups is statistically significant, p =0.006. However, there was found no statistically significant correlation between the status of the DTC in the BM and the status of the surgical resection margins (p = 0.278) (Table 5).

Table 5 The presence of DTC in the BM in patients with PC depending on the surgical resection margins

Surgical resection margins	DTC in BM (-) (n=59)		DTC in BM (+) (n=43)	
	n	%	n	%
Negative	43	72,9	27	73,8
Positive	16	27,1	16	26,2

Note: * - the difference between the groups is statistically significant, p =0.278.

It is important to assess the baseline PSA in relation to the status of the DTC in the BM. It was determined that PSA median in patients without DTC in BM (-) was 13 ng/ml (from 4 to 45 ng/ml), in the group of patients with DTC in KM (+) - 17.3 ng/ml (from 7 to 57 ng/ml) (Fig. 1).

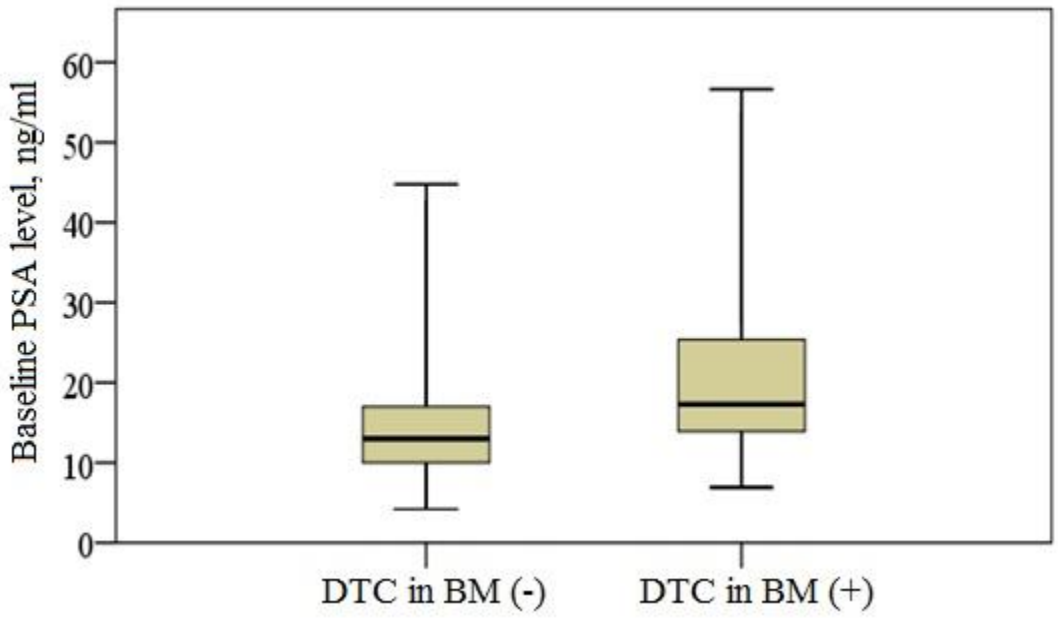

Fig. 1. Evaluation of the depending on the presence of the DTC in the BM

Distribution of patients by the range of baseline PSA and their statistical relationship depending on the presence of DTC in the BM is presented in Table 6.

Table 6 The presence of DTC in the BM of patients with PC depending on the baseline PSA level

Baseline PSA	DTC in BM (-) (n=74)		DTC in BM (+) (n=61)	
	n	%	n	%
<4 ng/ml	2	2.7	0	0.0
4-10 ng/ml	14	18.9	3	4.9
>10 ng/ml	58	78.4	58	95.1

Note: * - the difference between the groups is statistically significant, p =0.019

Thus, considering these patterns, it seems logical to evaluate the character of the disease course depending on DTC in the CM as a potential prognostic marker. An analysis was performed between this marker, the variants of primary treatment and the clinical status, i.e. remission or progression of the patients during the observation period (36 months). Additionally, we estimated survival rate of patients after primary treatment and the role of radical surgical treatment in the course of the disease in patients with different status of DTC in the BM.

Assessing the clinical course of the disease of all patients depending on the status of DTC in the BM revealed a correlation between the frequency of their detection and the progression of the disease (Table 7). The proportion of patients with remission of the disease among patients with DTC in the BM was 37.3%, and those with a progression of the disease -51.4%.

Table 7 Dependence of the rate of progression of the disease on the status of DTC in the BM

DTC in BM	Patients with remission (n=86)		Patients with progression (n=49)	
	n	%	n	%
-	57	62.7	17	48.6
+	29	37.3	32	51.4*

Note: * - the difference between the groups is statistically significant, p =0.001

The same pattern was found in the analysis of overall survival of patients (Table 8). The proportion of patients who were alive at the time of the end of the observation period (36 months) with DTC in the BM was 39.5%, and the mortality was 87.5%. The revealed relation was considered statistically significant (p = 0.001).

Table 8 Dependence of general survival on the status of DTC in the BM

DTC in BM	Patients who survived three years (n=119)		Patients who died (n=16)	
	n	%	n	%
-	72	60.5	2	12.5
+	47	39.5	14	87.5*

Note: * - the difference between the groups is statistically significant, p =0.001

Actuarial curves of recurrence-free and overall survival of patients with PC by Kaplan-Mayer, depending on the presence or absence of DTC in the BM are presented in Fig. 2.

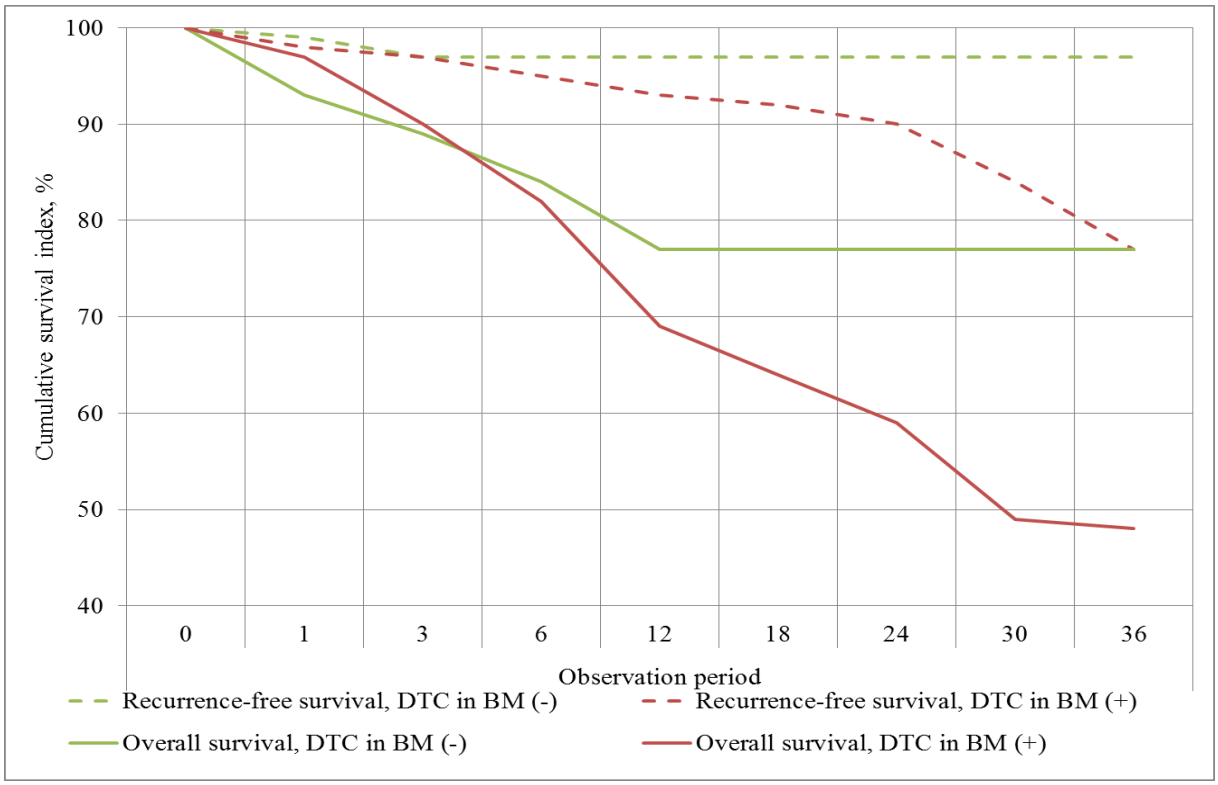

Fig. 2. Actuarial curves of recurrence-free and overall survival of patients with PC by Kaplan-Mayer

The prevalence of patients with existing DTC in the BM in the group of patients with conservative treatment by 12.3% was not significant. Analysis of a similar pattern in patients who have undergone radical surgical treatment revealed no correlation between the presence of DTC in the BM and the course of the disease. The proportion of patients with DTC in the BM did not differ significantly, as in patients who were in remission (25 patients, 37.3%), and in patients who were in a state of progression of the disease (18 patients, 51.4%).

The proportion of patients with DTC in the BM among the patients who were alive at the time of the end of the observation period was 41.2% (40 patients) and did not differ significantly from the proportion of patients who died (3 patients, 60%) (Fig. 3).

This could not be stated in patients of the second group, which were offered a variant of conservative treatment. The proportion of patients with a progression of the disease with an existing DTC in the BM was significantly higher (14 patients, 77.8%) compared to patients who were in remission (4 patients, 22.2%) (Fig. 3). This pattern was found to be statistically significant by the nonparametric Pearson criterion (the differences between the groups were significant, p = 0.001).

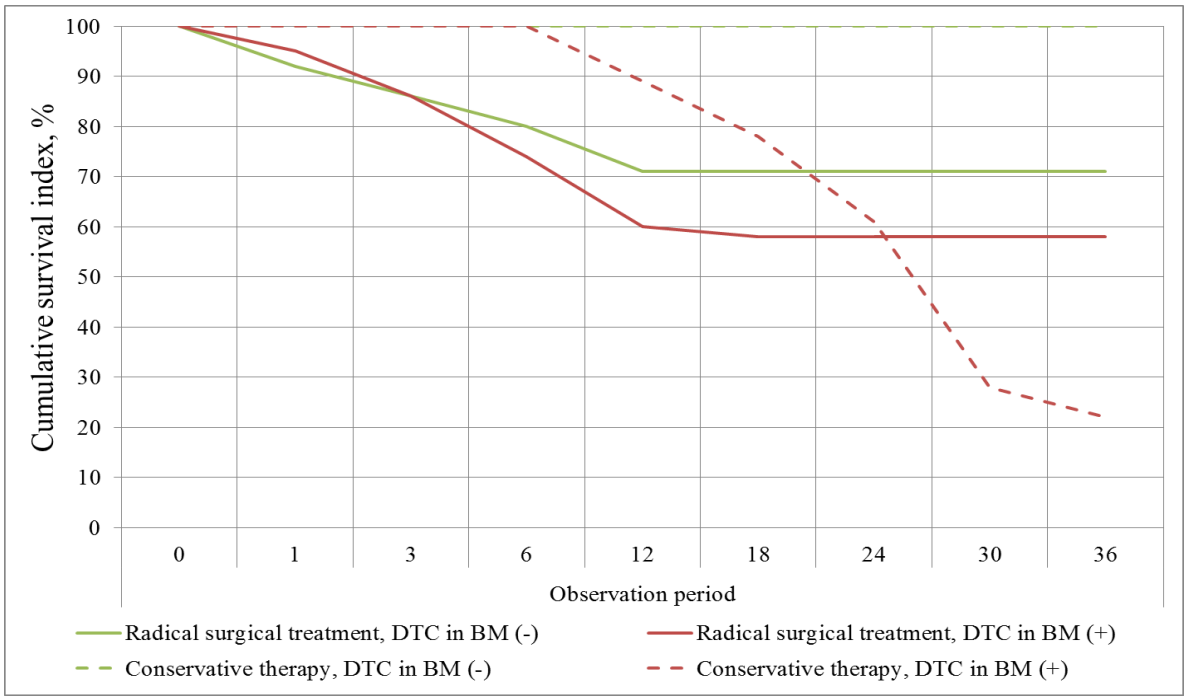

Fig. 3. Actuarial curves of recurrence-free survival of patients with PC by Kaplan-Mayer depending on the type of primary treatment

A similar pattern was obtained in analyzing the presence of DTC in the BM as a factor of negative effect on survival in patients after conservative therapy. The proportion of patients who died with the existing DTC in the BM was significantly higher (11 patients, 100%) compared with those who were alive at the end of the observation period (36 months) (7 patients, 31.8%) (Fig. 4).

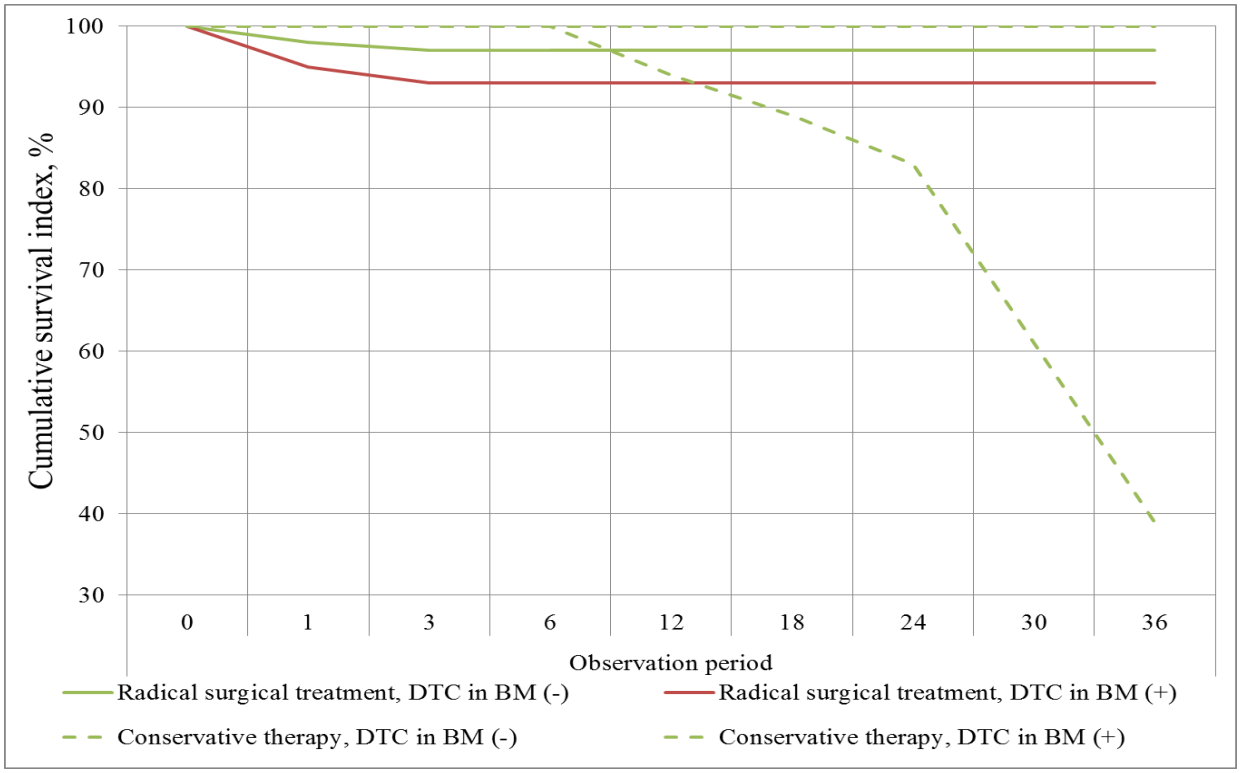

Fig. 4. Actuarial curves of overall survival of patients with PC by Kaplan-Mayer depending on the type of primary treatment

Thus, the effect of radical surgical treatment on the results of therapy in the distant period looks logical. The presence of DTC in the BM should be considered as a valid prognostic marker of an unfavorable clinical course of the disease in patients with PC, the behavior of which correlates with such known generally accepted markers as the stage of the disease, grading by the Gleason score, the baseline PSA level. However, the option of primary treatment can to some extent neutralize the negative impact of this marker on the course of the disease.

CONCLUSION

1. The presence of DTC in the BM of patients with PC should be considered as a marker of unfavorable prognosis of the disease course.

2. The status of the DTC in the BM has a close correlation with known and recognized markers of the prognosis of the disease, such as the stage of the disease, grading on the Gleason score, the baseline PSA level.

3. Radical surgical treatment of patients with PC can counter-balance the negative effect of DTC in the BM on the course of the disease, namely on patient survival and progression of the tumor process.

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