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FAST TRACK

Impact of disseminated tumor cells in bone marrow at diagnosis in patients with nonmetastatic prostate cancer treated by definitive radiotherapy

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The purpose of this study was to explore whether detection of disseminated tumor cells (DTCs) in bone marrow (BM) of nonmetastatic prostate cancer (PC) was associated with other clinical or histopathological factors at diagnoses or clinical outcome subsequent to definitive radiotherapy (RT). We evaluated BM aspirates from 272 $cT_{1\text{-}4}pN_0M_0$ PC patients by immunocytochemistry employing anticytokeratin antibodies (AE1/AE3). BM-status was compared with clinical and histopathological parameters. Long-term clinical outcome was assessed in 131 of the patients who all had completed definitive RT with or without androgen deprivation (AD), initiating treatment >5 years before cut-off date June 1, 2005. They had at least 1 unfavorable prognostic feature defined as $cT_{3\text{-}4}$ or Gleason score (GS) ≥7B or PSA ≥10 μg/l. Overall death, cause-specific death, distant metastases (DM) as first clinical relapse, local failure as first clinical relapse and biochemical failure were defined as end-points. DTCs were detected in 18% of the patients and were associated with increasing GS ($p = 0.04$) and percentage of Gleason pattern 4/5 ($p = 0.04$). The 7-year cumulative risk of DM was 21% for BM-positive patients *vs.* 6% for BM-negative patients ($p = 0.07$). In patients receiving RT without AD ($n = 75$), the 7-year cumulative risk of DM for BM-positive patients was 28% *vs.* 9% for BM-negative patients ($p = 0.03$). BM-status did not have impact on other end-points. In conclusion our study shows that presence of DTCs in BM at diagnosis was associated with the histological differentiation of the primary tumor and an increased risk of developing distant metastases after RT.

Key words: prostate cancer; minimal residual disease; immunochemistry; bone marrow; radiotherapy

The clinical course of prostate cancer (PC) varies from indolent to highly aggressive. Clinical TNM stage, histopathological Gleason score (GS) and prostate-specific antigen (PSA) are established prognostic factors for groups of patients with PC,[1] but are insufficient in predicting the individual course of the disease. Curative treatment strategies in patients with localized or locally advanced PC attempt to eradicate the tumor either by radical prostatectomy or radiotherapy (RT). Neoadjuvant and/or adjuvant androgen depression (AD) therapy is given to selected groups.[2] However, 5-year biochemical progression-free rates do not exceed 60–80% in patients with various unfavorable prognostic factors.[3,4] The lack of additional tools to predict the behavior of an individual tumor and its response to therapy, challenges patients and clinicians in their choice between surveillance and active treatment and hamper treatment consensus.

Development of distant metastases (DM), mainly skeletal, is the main cause of morbidity and a herald of mortality in PC patients. To improve the biological understanding of the metastatic process, sensitive immunocytochemical and molecular assays have been developed to detect single disseminated tumor cells (DTCs).[5] The presence of DTCs in bone marrow (BM) detected by immunocytochemistry is proven to be an independent prognostic factor in patients with breast cancer.[6] The method also yields prognostic information in other solid tumors like colorectal cancer,[7] lung cancer,[8,9] malignant melanoma[10] and osteosarcoma.[11] In PC, we previously demonstrated in a smaller series that finding of DTCs in BM at least 2 years after definitive external beam RT was associated with clinical progression.[12] However, this study failed to show any clinical prognostic significance of BM-status analyzed before treatment, possibly because of a short time of follow-up.

The present study had two aims: to explore whether DTCs in BM at diagnosis of nonmetastatic PC was associated with established clinical and histopathological features, and to retrospectively assess if larger sample-size and longer follow-up would confer any prognostic impact of such cells in patients treated by definitive RT.

Material and methods

Patients and pretreatment characteristics

Between 1994 and 2004, patients with newly diagnosed $T_{1\text{-}4}pN_0M_0$ prostate cancer as their first malignancy referred to Department of Radiotherapy at the Norwegian Radium Hospital (NRH, currently Rikshospitalet-Radiumhospitalet Medical Center), were consecutively asked to donate BM for detection of DTCs. The Regional Ethics Committee had approved the research project and all patients gave written informed consent. We retrospectively identified all patients who had donated BM in the time-period. This resulted in inclusion of 272 patients for the current analyzes.

The Tumor Node Metastases (TNM) system[13] was applied for patient classification. The cT classification was assessed by digital rectal examination and was in the present study dichotomized into $cT_{1\text{-}2}$ vs. $cT_{3\text{-}4}$. The pN_0 classification had been verified by pelvic lymphadenectomy, except for in patients who, based on their PSA <10 μg/l, according to the hospital's guidelines were regarded to be without regional lymph-node metastases. Pretreatment PSA was determined by an in-house time-resolved fluoroimmunometric assay[14] and for the purpose of this study dichotomized, the cut-off

Abbreviations: AD, androgen deprivation; BM, bone marrow; BF, biochemical failure; CI, confidence interval; DM, distant metastases; DTCs, disseminated tumor cells; GS, Gleason score; NRH, Norwegian Radium Hospital; MNCs, mononuclear cells; PC, prostate cancer; %G4/5, percentage of Gleason pattern 4/5; %TL, percentage tumor length in all cores; PSA, prostate-specific antigen; RT, radiotherapy; RT-PCR, real-time polymerase chain reaction.

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value being 10 μg/l. The M classification was evaluated by technetium bone scan and chest X-ray in all patients with supplementary skeleton X-ray or magnetic resonance imaging when indicated.

At referral, all PC diagnoses had routinely been confirmed by histopathological review of the patients' biopsies at the Department of Pathology, NRH. One experienced pathologist (A.B.) reassessed representative pretreatment biopsies for GS according to newly recommended guidelines[15] for the purpose of this study. In 10 patients, the biopsies were unavailable. In 5 of these, GS was assessed at diagnosis and this was taken into account. The last 5 were registered with unknown GS. GS was dichotomized into $\leq$7A (3 + 4) *vs.* $\geq$7B (4 + 3), as previously recorded.[16]

For available needle biopsies (n = 239), but not biopsies obtained by transurethral resection, the histopathological parameters "percentage of Gleason pattern 4/5" (%G4/5), "percentage positive biopsies" and "percentage tumor length in all cores" (%TL) were assessed. When there were 2 foci of cancer in the same needle, the uninvolved tissue between the foci was included in the assessment of %TL.

Bone marrow collection, preparation and immunostaining

Before start of any anticancer treatment, including hormone therapy, 20–30 ml BM was aspirated unilaterally from the posterior iliac crest following a 5-mm skin incision during local anesthesia. Two samples could not be processed because of coagulation during aspiration. The remaining 270 were processed as previously described.[17,18] Briefly, BM aspirates were diluted 1:1 in PBS (Life Technologies, Roskilde, Denmark). Mononuclear cells (MNCs) were isolated by density centrifugation using Lymphoprep (Nycomed, Oslo, Norway) and washed twice in PBS with 10% FCS (Biological Industries, Kibbutz Beit Haemek, Israel). Cytospins were made by centrifuging the MNCs down to polylysine-coated glass slides, each containing 5×10^5 cells. The slides were air-dried overnight and stored at -80°C until immunostaining was performed. Before immunostaining, the cytospins were fixed for 10 min in acetone. Four cytospins were then incubated for 30 min in a moist chamber, with the pancytokeratin mononuclear antibodies AE1/AE3 (Sanbio, Uden, The Netherlands), 1.1 μg/ml each. The visualization step included the alkaline-phosphatase/anti-alkaline-phosphatase reaction, and the slides were counterstained with hematoxylin to visualize nuclear morphology. Negative controls were performed in cytokeratin-positive patient samples by incubating additional cytospins with an isotype-specific irrelevant control monoclonal antibody. In 1999, the number of slides tested for negative control was increased from 1 to 4, as a consequence of methodological investigations.[17] In our study, 137 of the 270 samples (51%) were analyzed before this routine-change.

Detection of disseminated tumor cells

The cytospins were manually screened for immunostained cells by light microscopy using the 10× lens. To distinguish tumor cells from immunostained hematopoietic cells, all stained cells were according to published guidelines[19] closely evaluated by one experienced pathologist (J.M.N.), leading to a final conclusion of the BM-status to be either positive or negative of DTCs. The presence of positive cells classified as tumor cells in both the AE1/AE3 slides and in the corresponding negative control slides resulted in exclusion of the sample from any diagnostic conclusion. None of the samples analyzed before 1999, but 4 of the samples analyzed after 1999 (3.6%) were excluded, leaving 266 patients with a conclusive BM sample. The results of BM analyzes were not given to the clinicians deciding therapy.

Study design

In the first part of the study, all 266 conclusive pretreatment BM samples were compared with clinical parameters at diagnosis and histopathological features of the primary tumor. Thereafter, a subgroup of 131 patients were selected for follow-up analyzes based on the following criteria: (*i*) completion of definitive RT, (*ii*) treatment start at least 5 years before the study's cut-off date June 1, 2005 and (*iii*) at least 1 unfavorable prognostic factor at diagnosis defined as $cT_{3\text{-}4}$ or PSA $\geq$10 μg/l or GS $\geq$7B. Subset analyzes were performed on patients who received RT without AD.

Treatment

All the 131 patients who were eligible for follow-up analyzes received RT applied by a 4-field box technique as previously reported.[20] All patients treated before March 1996 (n = 50) received 66Gy without AD. Thereafter, the dosage was increased to 70 Gy and those with extracapsular growth ($cT_{3\text{-}4}$) or PSA >20 μg/l or GS >7 (n = 56) underwent neoadjuvant and adjuvant AD. The hospital's standard treatment at that time consisted of GnRh-analouge applied for 2 years. Three months were given neoadjuvant, the 1st month in combination with antiandrogen to avoid flare symptoms. This regime was given to 14 patients. The remaining 42 patients were included in the 7th trial of the Scandinavian Prostate Cancer Group. They received 3 months of neoadjuvant total androgen blockade followed by RT and life-long treatment with an oral nonsteroidal antiandrogen.[21]

Follow-up

Following primary treatment, the patients were generally followed up by their local urologist, but most patients additionally visited NRH one or more times during follow-up. The type and frequency of clinical examinations were left to the responsible clinician and for most patients clinical symptoms and not PSA-rise alone initiated diagnostic investigations for suspected tumor progression. To determine the latest survival status, cause of death, date and type of clinical progression and PSA measurements, the local physician was contacted and the hospital's records were reviewed. Clinical end-points were registered based on the latest available information before cut-off date June 1, 2005.

End-points

Overall death was defined as death due to any cause. Cause-specific death was defined as death due to PC, death due to complications from treatment of PC or death from unknown causes in patients who had been proven to suffer from progressive PC. Two types of clinical failure were defined: DM was defined as radiological evidence of distant spread and local failure as clinically evident growth of the prostate gland or locoregional lymph nodes. In accordance with the Houston definition, biochemical failure (BF) was defined as a PSA rise of at least 2 μg/l greater than the nadir (defined as the last nonrising value).[22] In addition, any secondary therapeutic intervention based on PSA-rise before the criteria was fulfilled was scored as BF at the time of intervention. The Houston criteria has been shown to provide superior accuracy in predicting subsequent clinical recurrence after RT with or without AD, compared to the ASTRO criteria and other definitions.[23–25]

Statistics

Statistical analyzes were performed applying the Statistical Package for Social Science (SPSS for windows 12.0, SPSS, Chicago, IL). The association between BM-status and clinical and histopathological factors were analyzed by χ^2 test (linear by linear association) for dichotomized variables and the Mann-Whitney test for continuous variables. The impact of BM-status on cumulative probability to encounter the clinical end-points was assessed by Kaplan-Meier curves. The observation time ranged from start of radiotherapy (or neoadjuvant AD when such was given) to the date of the end point, the patient's death or with the following modification the date of the last available clinical information before the study's cut-off date. When assessing the risk of DM, patients with preceding local failure were censored at the time of that clinical failure. Accordingly, when evaluating the probability of local failure, patients with DM prior to local failure were censored at the date when DM was detected. Consequently, only the first type of clinical relapse was registered as an event. The signifi-

TABLE I – PRETREATMENT CHARACTERISTICS

Variable	All patients (n = 266)	Follow-up cohort (n = 131)
Age in years, median (range)	66 (50–74)	66 (50–74)
<66 years	118 (44%)	56 (43%)
Clinical T classification		
cT_{1-2}	68 (26%)	28 (21%)
cT_{3-4}	198 (74%)	103 (79%)
PSA, mean (SD)	23 (17)	25 (17)
<10 µg/l	58 (22%)	25 (19%)
≥10 µg/l	208 (78%)	106 (81%)
Gleason score		
≤7A (3+4)	120 (45%)	54 (41%)
≥7B (4+3)	141 (53%)	73 (56%)
Unknown	5 (2%)	4 (3%)
% of Gleason pattern 4/5, mean (SD)	52% (39)[1]	57% (39)[2]
% positive biopsies, mean (SD)	71% (27)[1]	77% (25)[2]
% tumor length, mean (SD)	50% (30)[1]	56% (30)[2]

PSA, prostate specific antigen; SD, standard deviation.
[1]n = 239.–[2]n = 116.

TABLE II – DETECTION OF DISSEMINATED TUMOR CELLS IN BONE MARROW AT DIAGNOSIS COMPARED WITH PRETREATMENT CHARACTERISTICS (ALL PATIENTS, N = 266)

Dichotomized variables	No. of positive/ total no. of patients	%BM-positive patients	p[1]
Age			
<66 years	20/118	16.9	0.68
≥66 years	28/148	18.9	
Clinical T classification			
cT_{1-2}	9/68	13.2	0.23
cT_{3-4}	39/198	19.7	
PSA			
<10µg/l	12/58	20.7	0.55
≥10µg/l	36/208	17.3	
Gleason score[2]			
≤7A (3+4)	18/120	14.9	0.30
≥7B (4+3)	28/141	19.9	
Ordered categorical/ continuous variables	Mean rank BM-positive patients	Mean rank BM-negative patients	p[3]
Gleason score[2]	150	126	0.04
% of Gleason pattern 4/5[4]	142	116	0.04
% positive biopsies[4]	126	118	0.55
% tumor length[4]	123	120	0.81

BM, bone marrow; PSA, prostate-specific antigen.
[1]χ^2 test (linear by linear association).–[2]n = 261.–[3]Mann-Whitney test.–[4]n = 239.

cance of differences between survival curves was assessed by the log-rank test. Cox proportional hazards regression was applied to analyze the predictive impact of different factors in univariate and multivariate analyzes. Factors with $p < 0.1$ in the univariate analysis were selected for a backward conditional multivariate Cox analysis. All tests were 2-sided. Test results were considered to be statistically significant when $p < 0.05$.

Results

Pretreatment characteristics

Pretreatment characteristics for all 266 patients (and the follow-up cohort) are outlined in Table I. DTCs were present in BM of 48 (18%) of the patients. Positive BM-status was significantly associated with increasing %G4/5 (p = 0.04) and increasing GS (p = 0.04, Fig. 1). No associations were found with clinical or other assessed histopathological features (Table II). One patient had 11 DTCs/2 million MNCs, 2 had 4 positive, 6 had 3 positive, 9 had 2 and 30 had 1 MNC/2 million MNCs.

Follow-up analyzes

The 131 patients who were eligible for the follow-up analyzes had a median observation time of 7 years (range 1–10 years for all and 5–10 years for surviving patients). The BM was positive for DTCs in 26 (20%) of them. The mean radiation dose was 68.5 Gy (range 66–72 Gy). During the observation period, 23 patients (18%) died, 12 of them (9%) because of prostate cancer. Twelve patients (9%) developed DM as first clinical relapse and 8 patients (6%) were diagnosed with local failure as first clinical relapse. Out of 130 patients with available PSA results, 61 (47%) fulfilled the chosen definition of BF. After a 7-year observation period, the presence of DTCs tended to predict the development of DM as first clinical relapse but was not associated with any other endpoints of failure (Table III). The 7-year cumulative risk of DM as first clinical relapse was 21% for BM-positive patients vs. 6% for BM-negative patients (p = 0.07) (Fig. 2*a*). There were no difference in clinical outcome between patients with one DTC/2 mill MNCs and those with more than one DTC/2 mill MNCs (data not shown).

In patients with GS ≥7B, the 7-year cumulative risk of DM as first clinical relapse was 34% for those with positive BM-status compared to 10% for BM-negative patients (p = 0.04) (Fig. 2*b*). The BM-status was not associated with development of DM in patients with GS ≤7A (Fig. 2*b*).

Out of the 75 patients who were treated with RT without AD, the 7-years cumulative risk of DM as first clinical relapse was 28% for BM-positive patients and 9% for BM-negatives (p =

TABLE III – CLINICAL IMPACT OF BONE MARROW STATUS (KAPLAN-MEIER METHOD)

End point	Bone marrow status	% 7-years cumulative risk of failure		p (log-rank)	
		All patients (n = 131)	RT mono (n = 75)	All patients (n = 131)	RT mono (n = 75)
Overall death	Negative	14	15	0.70	0.72
	Positive	13	13		
Cause-specific death	Negative	8	9	0.72	0.99
	Positive	10	7		
Distant metastases as first clinical relapse	Negative	6	9	0.07	0.03
	Positive	21	28		
Local failure as first clinical relapse	Negative	6	8	0.61	0.25
	Positive	4	0		
Biochemical failure (n = 130)	Negative[1]	60	76	0.51	0.91
	Positive	36	66		

RT mono; Radiotherapy without androgen depression.
Negative bone marrow status: n = 105 out of all patients and 60 out of the 75 patients in the RT mono group expect [1]n = 104 and 59 (1 patient who suffered from early distant relapse and death could not be analyzed for BF because of missing PSA-results).
Positive bone marrow status: n = 26 out of all patients and 15 out of the 75 patients in the RT mono group.

0.03) (Fig 3). Also in this group, DTCs were not associated with any other end-points (Table III).

Univariate and multivariate analyzes

Table IV outlines the results from Cox regression univariate analyzes of different prognostic factors' impact on DM in all 131 patients. PSA was analyzed as a continuous variable because no patients with PSA <10 μg/l relapsed with DM. GS, PSA, BM-status, radiation dose and the presence or absence of AD-treatment were included in the a backward conditional Cox regression multivariate because of significant impact on development of DM in univariate analyzes ($p < 0.10$) (%G4/5 was excluded from the multivariate analysis despite of $p < 0.1$ because of considerable overlap with dichotomized GS). Radiation dose (RR = 5.93 [95% confidence interval (CI) = 1.28–27.57], $p = 0.02$), GS (RR = 6.15 [0.80−47.88], $p = 0.08$) and BM-status (RR = 3.34 [1.05−10.68], $p = 0.04$) remained in the final model of factors with independent impact on DM as first clinical relapse.

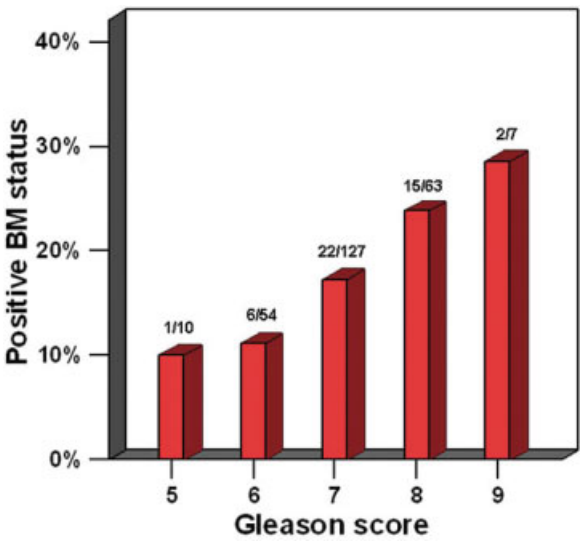

FIGURE 1 – The percentage of patients with disseminated tumor cells in bone marrow (BM) at diagnosis increased by increasing Gleason score of the primary tumor ($p = 0.04$, Mann-Whitney).

Discussion

The present study reports a significant association between immunocytochemically detected DTCs in BM at diagnosis of nonmetastatic PC and the histopathological differentiation of the primary tumor. In follow-up analyzes of patients with unfavorable prognostic factors and at least 5 years follow-up after definitive RT, we also observed that the presence of such cells significantly predicted development of DM in patients who did not receive any adjuvant treatment and in patients with GS ≥7B independent of AD. This is to our knowledge the first time an association is shown between DTCs in BM at diagnosis and manifest clinical relapse in radically treated PC patients.

In some contrast to previous studies which reported 24–54% prevalence of immunocytochemically detected DTCs in BM of nonmetastatic PC patients,[26–29] we found such cells in only 18% of the patients. Although not clear, the differences in prevalence of DTCs among these studies might be due to methodological aspects such as the use of different tumor associated antibodies and morphologic criteria for tumor cell identification. The previous studies have also yielded controversial results whether DTCs are associated to clinical and histopathological parameters. In the current study, we found a significant association between DTCs and both increasing %G4/5 and increasing GS, suggesting that tumors of high grade are more likely to shed tumor cells compared to low-grade tumors. The reason why we did not find any associations between DTCs and other well established prognostic factors such as T-classification and PSA-level is not clear. Most probably, only increased sample size will reveal if such associations exist.

In PC, the change from minimal residual disease to manifest metastases develops over a long time, probably due to dormancy where the cells rest in the G0 phase of the cell cycle.[30] Studies of localized PC therefore require many years of follow-up to provide a sufficient number of clinical events in order to demonstrate differences in survival. Biochemical progression is often applied as a surrogate marker for subsequent clinical relapse. Weckerman *et al*. reported that DTCs predicted BF in 82 prostatectomized patients after a median follow-up of 4 years.[29] In contrast, the presence of DTCs in BM did not predict BF in our study. The reason for this is not clear. One theoretical reason might be that while our study suggests that DTCs predict DM and not LF, BF does not distinguish between these different failures. Because of the suboptimal radiation-dose given, a substantial fraction of the patients with rising PSA are expected to have asymptomatic local growth, not yet to be diagnoses. Furthermore, only our group has so far published results regarding BM-status in PC-patients prior to RT,

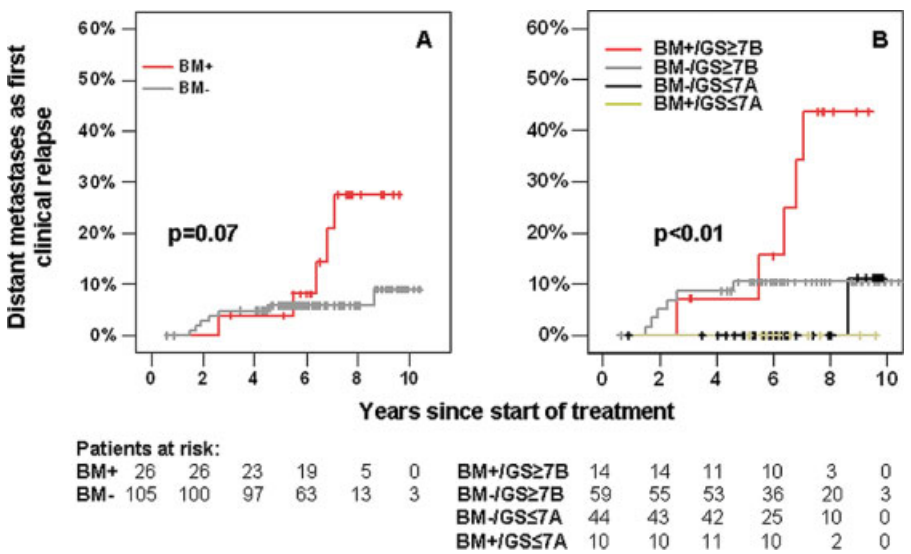

FIGURE 2 – Cumulative risk of developing distant metastases as first clinical relapse related to bone marrow status. (A) All patients in the follow-up cohort ($n = 131$). (B) Combination of Gleason score and bone marrow status (4 patients are excluded due to lacking Gleason score). BM+, positive bone marrow status; BM−, negative bone marrow status. Log-rank test for differences between curves in B: BM+/GS ≥ 7B *vs*. BM−/GS ≥ 7B; $p = 0.04$, BM+/GS ≥ 7B *vs*. BM+/GS ≤ 7A; $p = 0.08$, BM−/GS ≥ 7B *vs*. BM−/GS ≤ 7A; $p = 0.14$, BM−/GS ≤ 7A *vs*. BM+/GS ≤ 7A; $p = 0.64$.

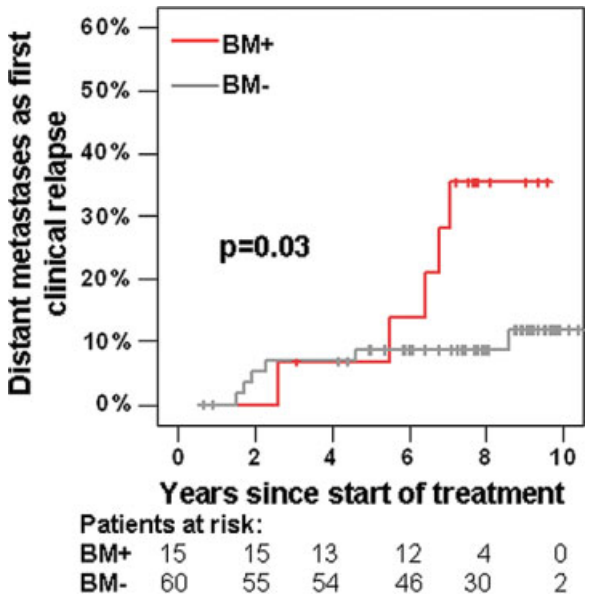

FIGURE 3 – Cumulative risk of developing distant metastases as first clinical relapse in patients who received definitive-dose radiotherapy without any neoadjuvant or adjuvant androgen depression ($n = 75$).

TABLE IV – CLINICAL AND HISTOPATHOLOGICAL PARAMETERS' IMPACT ON DEVELOPMENT OF DISTANT METASTASES AS FIRST CLINICAL RELAPSE IN 131 PATIENTS WITH NONMETASTATIC PROSTATE CANCER TREATED BY DEFINITIVE RADIOTHERAPY (UNIVARIATE COX REGRESSION ANALYSIS)

	RR (95% CI)	*p*
Dichotomized variables		
BM-status (negative/positive)	2.78 (0.89–8.80)	0.08
Age (<66/≥66)	1.53 (0.46–5.08)	0.49
T category ($cT_{1\text{-}2}/cT_{3\text{-}4}$)	1.68 (0.37–7.74)	0.51
Gleason score (≤7A/≥7B)[1]	7.77 (0.99–59.47)	0.05
Radiation dose (70Gy/<70Gy)	6.77 (1.45–31.69)	0.02
Adjuvant AD/no AD[2]	5.79 (0.73–46.33)	0.10
Continuous variables		
PSA	1.03 (1.00–1.05)	0.03
% of Gleason pattern 4/5[3]	1.02 (1.00–1.05)	0.05
% positive biopsies[3]	1.02 (0.99–1.05)	0.15
% tumor length[3]	1.01 (0.99–1.03)	0.32

RR, relative risk; CI, confidence interval; BM, bone marrow; AD, androgen depression; PSA, prostate specific antigen.
[1]$n = 127$.–[2]As part of primary treatment.–[3]$n = 116$.

and because the prostate is not removed, BF in irradiated patients cannot be directly compared with BF after prostatectomy.

In the current study, the 7-year median follow-up enabled us to apply DM as a clinically more reliable end-point. For all the 131 patients in the follow-up cohort, we found a weak association between BM-status and DM. Interestingly, this relation emerged first after 7 years. Among patients who received RT without AD, BM-positive patients had a significant higher risk of developing DM as first clinical relapse compared to BM-negative patients. These results indicate that the method might be useful in decision making regarding neoadjuvant/adjuvant therapy. Admittedly, our radiation dose was suboptimal according to current standards.[31,32] The question remains open whether similar results can be achieved in patients receiving higher doses.

As the 2-tiered Gleason grouping earlier has been shown to be a very good discriminator of univariate failure-free survival in patients with locally advanced tumors,[16] it was of particular interest to notice that among patients with GS ≥7B, the 7-year cumulative risk of DM as first clinical relapse was 34% if DTCs were present in BM at diagnosis and only 10% for the BM-negative patients. Among the 10 BM-positive patients with GS ≤7A, none developed DM during the observation period. It cannot be ruled out that longer follow-up could reveal association between DTCs and clinical outcome also in patients with low-grade tumors. Nevertheless, this interesting finding leads to the question why not all patients with DTCs at diagnosis of localized cancer suffer from distant relapse. In fact, it has been shown that only proliferating DTCs predict relapse[33] and that DTCs are more likely to proliferate in patients whose tumors have high GS.[34] It is also possible that other factors such as cytogenetical aberrations associated with GS[35,36] are crucial for the single cells to form metastases. Furthermore, we cannot expect all DTCs to have clonogenic potential. According to the theory of cancer stem cells, only a few of the tumor cells, with the ability of self-renewal, have tumor-initiating properties.[37] This implies that only DTCs with such properties can develop into overt metastases.

Several investigators have identified DTCs in PC patients by real-time polymerase chain reaction (RT-PCR) in blood and BM. These studies have yielded controversial results with regard to the prevalence of DTCs and the prognostic value following prostatectomy.[38] Because of significant methodological problems, mainly due to low specificity,[39,40] our hospital has not favorized RT-PCR as a detection method for DTCs. However, detection of DTCs applying immunocytochemistry and tumor associated antibodies also has several methodological aspects. Efforts have therefore been done in order to standardize such procedures. Nevertheless, in the present study, we cannot exclude that lack of sufficient number of negative controls before 1999 might have resulted in positive scoring of patients who should have been excluded due to positive tests both in specific test and the negative control. Furthermore, the detection method applied in this study has recently been shown to give a 4% false-positive specific staining in BM from 98 healthy female donors.[41] The interpretation of the results is complicated by the fact that 43% of the patients in the follow-up cohort received AD as part of the primary therapy, and that most of these continued life-long antiandrogen treatment. AD might theoretically eradicate the DTCs and therefore overshadow their biological effects. This might explain why the association between DTCs and development of DM was stronger in the subset of patients who not received AD compared to all patients.

Monitoring of DTCs before and after therapy appears to be of clinical value both in breast cancer[42] and in PC[12] and should therefore be included in future trials. In accordance with the pilot study by Pantel *et al.*[43] showing that the presence of DTCs is reduced during AD, BM should be sampled in PC patients both before and after neoadjuvant AD. Such monitoring might clarify whether persistence of DTCs during AD gives early evidence of hormone-resistant disease in patients who could benefit from early alternative treatment modalities such as cancer vaccines[44] or chemotherapy.[45,46]

In summary, we have demonstrated an association between DTCs in BM at diagnosis of nonmetastatic PC and the histological differentiation of the primary tumor. Furthermore, our findings define a group of patients with DTCs in BM at diagnosis and GS ≥7B who have a particularly high risk of developing distant metastases following definitive RT. Although our data identifies important and interesting biological features of localized/locally advanced PC, a larger randomized study addressing the usefulness of DTCs as a biomarker in relation to androgen deprivation and dose escalation is necessary to be carried out before our findings can be implemented in clinical practice.

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