Prognostic Value of a Bone Marrow Scan Before and During Treatment of Bone Metastases With Radium-223-dichloride in Metastatic Castration-resistant Prostate Cancer

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Abstract

Background: Due to the risks of hematological side effects resp. clinical aggravation, not all patients with metastasized castration resistant prostate cancer (mCRPC) and bone metastases should undergo all six cycles of radium-223-dichloride therapy. A parameter helping us to assess the chances of successful therapy management as well as the risk of hematological side effects is needed. The aim of this study is to analyze “Bone Marrow Scintigraphy” (BMS) as a prognostic factor.

Methods: 106 patients were included in our single-center study. 93 patients underwent BMS with 492-667 MBq Tc-99m Scintimun® prior to therapy. The “Uptake Ratio” (UR) was calculated by positioning “regions of interest” (ROI) around the sacroiliac region, the sternum and the background areas. We calculated an UR threshold value by means of a “Receiver-Operating-Characteristics” (ROC) curve. The UR and the “Bone Scan Index” (BSI), which is automatically gained by bone scintigraphy, were related and compared to other measured parameters (lab parameters, androgen deprivation therapy (ADT)). Statistics software IBM® SPSS® Statistics (Version 24.0) was employed to identify prognostic factors by univariate und multivariate analyzes.

Results: The sacroiliac area-to-background technique proved helpful in combination with the calculated threshold UR=8 for quantitative assessment of a BMS. Radium-223-dichloride treatment showed significantly lower hematological toxicity at UR>8 (p=0.001). More precisely subdivided, there was a significant effect on to the number of erythrocytes (p=0.018), leucocytes (p=0.030) und neutrophil granulocytes (p=0.031). Significant prognostic factors influencing the “Overall survival” (OS) were the bone marrow (BM) distribution pattern (p=0.039) and the calculated UR (p=0.027).

Conclusion: An UR value of 8 can be used for an approximation in prognostic assessment of risks and chances of this therapy. A combination of a qualitative and a quantitative evaluation of pre-therapeutic BMS could help to minimize therapy drop-outs and an avoidable exposure to radium-223-dichloride-therapy as well as other potential radio-nuclide therapies.

Background

Both our own clinical experience and that of other clinical centers [1, 2] show that 35–50% of patients treated with radium-223-dichloride were not able to undergo all of the originally planned six cycles. The cause is associated with the varying physical condition of these patients with bone metastatic castration resistant prostate cancer (mCRPC), who are in a progressed state of this malignant disease [3, 4]. Individual treatment planning by means of pre-therapeutic dosimetry has not been standardized so far due to its complexity [5]. Most studies report therapy-induced effects on overall survival (OS) or the occurrence of skeletal events [1, 2, 6]. However, studies focusing on specific pretherapeutic diagnostics that can close the gap between progressing mCRPC and therapy initiation with radium-223-dichloride are missing. Infiltration and displacement of the functional bone marrow (BM) in the course of mCRPC impedes the concept of so-called “theranostics”, as predictive parameters for assessment of the residual

BM are missing. As a result, limited hematopoiesis and a reduced overall condition can lead to a break-off of potentially LQ-improving and life-prolonging therapies [7, 8]. In addition, previous chemotherapies and local external percutaneous radiation therapy (EBRT) of the prostate gland or osseous metastases lead to an increased vulnerability of the hemopoietic system [9, 10].

Due to the radium-223 alpha beam's limited range of < 100 µm, healthy tissue is only damaged to a minimal extent [11]. By the end of radium-223-dichloride therapy, the radioactive load applied to the hemopoietic BM is only 1.6 Gy in a patient of 70 kg body weight [12]. Radium-223-dichloride is known to show a low profile of myelosuppressive side effects. Nevertheless it can lead to anemia and thrombocytopenia [13]. In this respect, pretherapeutic bone marrow scintigraphy (BMS) could help to better identify the patient population affected by this. According to current guidelines, BMS is not part of the indication screening prior to treatment of symptomatic bone metastases with radium-223-dichloride [14].

BMS has been rendered nearly obsolete by new technologies like magnetic resonance imaging (MRI) over the past few decades [15]. Due to the lack of sensitive and specific quantification parameters in patients with mCRPC diagnostic analysis of BMS is merely visual, leading to great inter- and intra- observer variability. Munz et al. were the first to perform background-corrected uptake-measurements during BMS in the region-of-interest (ROI) technique using Tc-99m nano colloid ; this technique was supplemented by methodically modified further studies by the work groups around Huić et al. and Ivancévic et al., both working with Tc-99m-labelled monoclonal antibodies (MAB) [16–18]. This technique, however, was never implemented in clinical routine [16, 17]. One of the problems in using the formula devised by Munz resp. Huić et al. consists in its non-applicability after attendant medication. The study sets out to evaluate the prognostic value of pretherapeutic BMS in patients with mCRPC as well as to analyze the BM residue during radium-223-dichloride-therapy. We aim to focus especially on identifying a threshold for improved definition of the target population and thus to contribute to risk minimization and preservation of life quality.

Patients & Methods

Patients

In the period between January 2014 and February 2018, 106 patients with mCRPC (median age 72, range 48–89 years) were enrolled and treated with radium-223-dichloride (Xofigo®, Bayer) in a uni-center one-arm study at the University Hospital Schleswig-Holstein, Campus Kiel, Department of nuclear medicine. All 106 patients underwent bone scintigraphy prior to Xofigo®-therapy and 93 patients underwent additional BMS. 86 patients (81%) were 65 years old or older. In 77 (73%) patients local EBRT of prostate gland or osseous metastases with a mean dose of 66 Gy had been performed earlier. All patients received radium-223-dichloride at a standard dose of 55 kBq/kg/KG in 4-week cycles, up to a maximum of 6 applications. Information on previous applications of systemic therapies including chemotherapy, e collected. The differential blood count was

assessed prior to each cycle with radium-223-dichloride. Other lab parameters, including prostate specific antigen (PSA) and alkaline phosphatase (AP), were assessed before and during treatment as well as during follow-up 3 months post-treatment. The scintigraphic data were compared with lymphocytes, erythrocytes, leucocytes, and neutrophil granulocytes (NG), thrombocytes, PSA, AP, lactate dehydrogenase (LDH) as well as hemoglobin (Hb), and statistically processed.

Imaging

Bone Scintigraphy

Due to the large number of bone scintigraphies performed outside our institution, not all raw data of 106 bone scintigraphies were available. Pre- resp. post-therapeutic bone scintigraphy was performed during the mineralization phase by means of a dual-head gamma camera (E-CAM/Symbia/Intevo, Siemens Healthcare, Erlangen, Germany) in 40 out of 44 patients. We employed a LEHR-collimator and an energy window of 15% centered on the 140-keV-Photopeak of Tc-99m. The thus generated raw data were subject to further analysis. Prior to bone scintigraphy we performed an intravenous injection of a bolus of 650–700 MBq (10 MBq/kg KG) Tc-99m-HDP. After 3 h planar (anterior, posterior) whole body scans were done in dorsal position with a matrix of 256 × 1024 pixels and a table progression of 10 cm/min. The collected data were processed with Syngo-VB10B-software (Siemens) and the following)protocol: “GK Skelett 3 h (E-CAM)/Skelett WB 3 h (Body-Scan)“.

Bone Marrow Scintigraphy (bms)

Approximately one month prior to therapy, planar BMS was performed in 93 patients after injection of 492–667 MBq Tc-99m-labeled anti-granulocytes monoclonal antibodies (Besilesomab, Scintimun®, CIS bio international, France). 3–4 hrs p.i. we acquired planar anterior and posterior body scans in dorsal position with a dual-head gamma camera in analogy to the bone scintigraphy configurations (cf. bone scintigraphy). As we were able to exclude previous BMS, we did not test for human anti-mouse antibodies (HAMAs). The here employed configuration MAB BW 250/183 is directed against the granulocyte antigen NCA-95 and binds to the surface of NG, promyelocytes, myelocytes and metamyelocytes resp. their pre-stages in the red BM [19]. The image shows a morphological correlative of the red bone marrow and thus of the bone marrow reserve (Fig. 1a-c).

Image Analysis

Bone Scan Index (BSI)

The percentage of bone mass affected by metastases was measured by calculating the BSI using an automated method [20]. This automated approach was evaluated by means of the commercially

available software EXINI bone™ (EXINI Diagnostic AB, Lund, Sweden). The software serves to analyze and assess the so-called hotspots by means of an algorithm. Hotspots with surplus nuclide accumulation are indicators of the bone metabolism intensity, such as may be causes by metastases or other findings that are correlated with an increased bone metabolism as for instance bone fractures (Fig. 1d).

Uptake-ratio Of Bms

Quantitative analysis of BMS was performed in a posterior view in the sacroiliac region and in the anterior view for sternal analysis. ROIs were positioned around the sacroiliac resp. the sternal region and in the background region between the left and the right Kidney, the spine and the pelvis. Both irregular and regular ROIs were marked (Fig. 1, a-c). The uptake ratio (UR) was calculated as follows according to the method by Munz et al. [16]:

$$UR = \frac{ROI\ bone\ marrow\ \left(\frac{counts}{pixel}\right) - ROI\ background\ \left(\frac{counts}{pixel}\right)}{ROI\ background\ \left(\frac{counts}{pixel}\right)}$$

The larger the value of UR, the higher the metabolic activity in the area of interest.

Statistical analysis

Statistical analysis was done using SPSS 24. A benchmark test with two or more variables was done by the Mann-Whitney-U test resp. the Kruskal-Wallis test. Deviations were regarded as “significant” if the significance level was < 0.05. The significance level was adjusted according to Bonferroni. Correlations were done after Pearson and checked for causality by variance and regression analyzes. A Receiver-Operating-Characteristics (ROC) analysis served to identify a threshold. Chi-square test was used for analysis of differences between the two patient groups. Overall survival (OS) was defined as the time from starting the treatment with radium-223-dichloride to the end of the study or date of death. OS of the patients was calculated by the Kaplan-Meier method, and the difference between the curves was analyzed by means of the log rank test. Factors associated with OS were evaluated by univariate and multivariate analyzes with the Cox proportional hazard method (forward and backward selection for patients with complete data sets). The level of significance was < 0.05. To investigate intraobserver variability in calculating UR as well as to compare between different methods, a subgroup of 10 BMS was analyzed 5 times under calculation of the intra-correlation coefficient (ICC).

Results

Bone Marrow uptake and metastatic load

The uptake of the sternum (r=-0.345; p = 0.027) respectively of the sacroiliac region (SIR) (r=-0.411; p = 0.008) showed a medium strong significant negative correlation with pretherapeutic BSI (BSI *pre*). This result supports the hypothesis that the bone marrow, in case of a high metastatic load, shows a limited reserve, or, in other words, that a reduced BM reserve as consequence of chemotherapy or radiotherapy indicates a predisposition for a high BSI [9, 21]. We found confirmation of a statistically significant difference between the BSI *pre* and the UR sternum ($p < 0.001$). Thus, a low BSI *pre* corresponded with a high BM uptake in the area of the sternum. The BM uptake of the SIR correlated slight with that of the sternum (r = 0.166; p = 0.013). A variance analysis confirmed significant differences between the determined UR-SIR and the UR-Sternum ($p < 0.001$). When comparing between the patients with/without radiotherapy resp. with/without chemotherapy, the test did not show any different tendencies of the applied ROI-techniques. The level of BSI *pre* had an impact on UR. When BSI *pre* is increased by one unit, UR-sternum decreases by 0.311 units (p = 0.027); UR-SIR deceases by 0.343 units respectively (p = 0.008).

Correlations With The Hemogram

The uptake calculated by the SIR-to-background-ratio showed a significant correlation according to Pearson with the biochemical parameters AP, LDH and PSA as well as with the hematological lab parameters Hb value, erythrocyte count, leucocyte count, NG and thrombocytes. Table 1 shows the results of the correlation analysis, the significant values appear as bold figures. The stronger the uptake of the central BM, the higher are the Hb values; respectively the higher the Hb, the higher the UR in SIR.

Table 1
Cycle-dependant correlations of the UR in relation to the hemogram

	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
	r (p-Value; n)					
AP						
UR-SIR	**-0.214 (0.040*;93)**	**-0.231 (0.027*; 91)**	**-0.285 (0.009**; 82)**	**-0.242 (0.044*; 70)**	-0.234 (0.059; 66)	-0.188 (0.155; 59)
UR-Sternum	-0.132 (0.206; 93)	-0.707 (0.509; 91)	-0.045 (0.688; 82)	-0.128 (0.289; 70)	-0.065 (0.606; 66)	-0.182 (0.168; 59)
LDH						
UR-SIR	**-0.210 (0.048*; 89)**	-0.157 (0.141; 89)	**-0.241 (0.030*; 81)**	-0.092 (0.450; 70)	-0.090 (0.474; 66)	-0.095 (0.476; 59)
UR-Sternum	-0.064 (0.550; 89)	-0.111 (0.300; 89)	-0.114 (0.312; 81)	-0.067 (0.583; 70)	-0.107 (0.392; 66)	-0.085 (0.524; 59)
PSA						
UR-SIR	0.056 (0.593; 93)	0.136 (0.199; 91)	-0.123 (0.269; 83)	**-0.244 (0.042*; 70)**	-0.214 (0.085; 66)	**-0.316 (0.015*; 59)**
UR-Sternum	-0.023 (0.829; 93)	0.013 (0.902; 91)	-0.136 (0.220; 83)	-0.117 (0.337; 70)	-0.041 (0.746; 66)	-0.056 (0.674; 59)
Hb						
UR-SIR	**0.345 (0.001**; 93)**	**0.364 (< 0.001***; 91)**	**0.323 (0.003**, 82)**	**0.274 (0.022*; 70)**	0.198 (0.114; 65)	**0.283 (0.030*; 59)**
UR-Sternum	0.112 (0.286; 93)	0.201 (0.056; 91)	0.139 (0.213; 82)	0.095 (0.433; 70)	0.098 (0.436; 65)	0.081 (0.540; 59)
Erythrocytes						
UR-SIR	**0.210 (0.044*; 93)**	**0.236 (0.024*; 91)**	0.147 (0.187; 82)	0.104 (0.391; 70)	0.031 (0.807; 65)	0.144 (0.277; 59)

AP-Alkaline phosphatase, Hb-Hemoglobin, LDH-Lactat dehydrogenase, PSA-Prostate specific antigen, BSI-Bone Scan Index, SIR-Region of Sacroiliac, NG-Neutrophil granulocytes, p-Value: *<0,05; **<0,01;

	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
	r (p-Value; n)					
UR-Sternum	0.074 (0.480; 93)	0.183 (0.082; 91)	0.097 (0.386; 82)	0.018 (0.885; 70)	0.030 (0.811; 65)	0.022 (0.868; 59)
Platelets						
UR-SIR	0.001 (0.996; 93)	0.123 (0.244; 91)	0.148 (0.185; 82)	0.155 (0.201; 70)	0.210 (0.093; 65)	**0.265 (0.042*; 59)**
UR-Sternum	0.005 (0.965; 93)	-0.006 (0.958; 91)	0.060 (0.592; 82)	-0.036 (0.768; 70)	0.045 (0.724; 65)	0.046 (0.727; 59)
NG						
UR-SIR	0.158 (0.155; 83)	0.149 (0.186; 80)	**0.267 (0.027*; 68)**	0.181 (0.156; 63)	0.067 (0.616; 58)	0.088 (0.544; 50)
UR-Sternum	0.012 (0.914; 83)	-0.016 (0.890; 80)	0.024 (0.843; 68)	0.009 (0.942; 63)	-0.064 (0.631; 58)	-0.085 (0.559; 50)

AP-Alkaline phosphatase, Hb-Hemoglobin, LDH-Lactat dehydrogenase, PSA-Prostate specific antigen, BSI-Bone Scan Index, SIR-Region of Sacroiliac, NG-Neutrophil granulocytes, p-Value: *<0,05; **<0,01; ***<0,005

In summary, when applying the ROI technique, BM uptake in SIR correlated with hematological markers, while BSI correlated with biochemical markers. Essentially, we can say that a high BSI correlates with a lower BM-Uptake both in SIR- as well as the sternum-to-background-analysis. As the definition of ROIs above the sternum may lead to overlays with the spine, the liver and possibly the spleen and in the absence of correlations with lab parameters, further analyzes were only done via the quantitative SIR-to-background measurement.

Classification Of The Bone Marrow Status

The uptake ratio was 0.22-24,02 at a median value of 7.86, rounded up to 8 for using integral numbers for this study. Compared to the group with 4–6 therapy cycles the median of patients with 1–3 applications show an UR that was lower by 1.2 units (p = 0.212). In total, 63 (59%) patients completed the entire 6 cycles.

Based on the determined cut-off of 8, the collective was divided in two groups. (Group 1 ≤ 8, Group 2 > 8). We devised a tabular division of valid BM-scintigraphy scans into BM-distribution groups I-V (cf. Table 2; modified acc. to Munz et al. [16]), taking into account both the visual analysis of the BM distribution

pattern, the number of focal lesions as well as quantitative graduation of UR. Graduation of UR is as follows: A (> 8) and B (≤ 8). Up to Group II the distribution showed a prevalence of higher UR. At higher grades of peripheral expansion, UR ≤ 8 was prevalent.

Table 2
Bone marrow status (modified and supplemented version based on a suggestion by Munz et al. 1984)

Group	BM-distribution pattern	Number of focal lesions	UR		Total n (%)
			A (> 8)	B (≤ 8)	
I	Normal	+	1	1	19 (21)
		++	7	1	
		+++	2	2	
		++++	1	4	
II	Moderate peripheral expansion	+	1	0	36 (39)
		++	5	3	
		+++	7	4	
		++++	9	7	
III	Marked peripheral expansion	++	1	1	24 (26)
		+++	0	1	
		++++	9	12	
IV	Displacement	++++	2	8	10 (11)
V	Non-visualization	++++	0	1	3 (3)
		0	0	2	
Total n (%)			45 (49)	47 (51)	92
+ = 1 lesions, ++ = 2–4 lesions, +++ = 5–10 lesions, ++++ = >10 lesions, 0 = not assessable, UR – Uptake ratio, BM – Bone marrow					

Defining A Threshold By Means Of The Roc Analysis

The area under the curve (AUC) was 0.678 (95% Confidential Interval (CI): 0.540–0.817) and was statistically significant (p = 0.014). The Threshold of 8 at a sensitivity of 0.714 (71%) and a specificity of

0.551 (55%) (1-specificity = 0.449) was determined as the best UR-cut-off value for prediction of the bone marrow-status.

Tetragonal And Polygonal Rois

Both ROI techniques, i.e. both tetragonal and polygonal ROI, were found adequate for analysis of BM uptake. Correlation between both procedures were highly significant with five comparative measurements at 0.843 to 0.998 ($p = 0.002$ to < 0.001). The Correlation within the classes was 0.923 at a significance level of < 0.0001.

Intraindividual Difference In The Roi Technique

Intraindividual differences occurring in tetragonal ROI-technique were negligible at an ICC of $r = 0.995$ ($p < 0.001$). Reliability analysis of the polygonal ROI technique showed a similar result at $r = 0.991$ and $p < 0.001$. Assessment with the Bland-Altman method showed that 95% of the values of both methods were within the thresholds 4.81 and 3.71.

Hematotoxicity During Therapy In Relation To Ur Status

At an UR status below resp. above 8 we observed discrepancies between the groups regarding the following parameters: AP value, LDH, Hb value, number of erythrocytes, leucocytes and NG.

Initially we considered the AP value in the serum. Pre-therapeutically, the median AP value at UR ≤ 8 was 144 U/l (95% CI: 113–200) and thus 42% higher than at UR > 8 (Median (M) = 83 U/l; 95% CI: 76–129). Testing for significance was positive ($p = 0.030$). Therefore, patients with UR > 8 were within the reference area of the AP value. In the third therapy cycle, the LDH value differed significantly in relation to UR ($p = 0.034$). By contrast the PSA value was not significantly different when comparing the groups. A BM reserve at UR > 8 was confirmed to have a favorable impact on the parameters AP and LDH.

No differences were found with respect to thrombocytes and lymphocytes between the groups. However, there were differences regarding Hb, both pre-therapeutically and prior to the third cycle with respect to UR ≤ /> 8. Prior to the first cycle UR ≤ 8 showed a median Hb value of 11.9 g/dl (95% CI: 11.4–1.4) and for UR > 8 the value was 13.2 g/dl (95% CI: 12.8–13.6). This is a difference of 11% ($p = 0.001$). Towards the third cycle the difference was slightly lower at 10%, but it remained significant ($p = 0.002$). Discrepancies in the Hb value were significant in relation to UR were also significant at the final therapy stage ($p = 0.040$) and during follow-up ($p = 0.031$). A similar trend could be observed regarding the number of NG. Pre-therapeutically, the number of NG was 4.00×10^9/l (95% CI: 3.19–4.52) at UR ≤ 8, and 4.41×10^9/l (95% CI: 3.76–5.58) at UR > 8 ($p = 0.031$). The effect was even stronger at a significant difference of 33% prior to the third radium-223-dichloride treatment ($p = 0.005$). In addition, there was a significant

 = 0.018) as well as a discrepancy in leucocytes

(p = 0.030) prior to radionuclide application. All of the afore-mentioned hematological parameters proved less hemotoxic at UR > 8.

Survival Analysis

The main issue of the study was to analyze the survival time and to evaluate prognostic factors. The total collective of the study achieved a median survival time of 14 months (95% CI: 8.62–19.37) after first time of treatment with radium-223 dichloride (Fig. 2a). A total of 106 patients were included in the survival analysis, 69 of these died by the end of the analysis period. Patients who completed 4–6 cycles showed a statistically significant survival advantage (p < 0.001). The median survival period difference was 14.2 months when comparing 4–6 cycles (M = 20.65; 95% CI: 16,67 − 19,37) with 1–3 cycles (M = 6.41; 95% CI: 2.62–10.19). As shown in Fig. 2b, patients with UR > 8 vs. those with UR ≤ 8 showed a statistically non-significant survival advantage of 5.8 months. The primary AP value also seems to have an impact on survival. Patients, with a primary AP value of > 129 U/l, had a statistically shorter life expectancy (M = 10.42; 95% CI: 8,72 − 12,11). Patients with an AP value ≤ 129 U/l lived 19.8 months (95% CI: 15,61 − 24,08) and thus 9.4 months longer. This difference was revealed to be statistically highly significant (p < 0.001) (Fig. 2b).

Cox-regression For Overall Survival

To test the prognostic accuracy of the survival we performed a Cox multivariate regression analysis. Table 3 shows the results of univariate analysis. 72 patients were included in total and all significant factors of the univariate analysis were included. The number of completed cycles (p < 0.001) as well as ADT or drug treatment with Abiraterone (p = 0.012), the BM distribution pattern (p = 0.020) and the PSA-response after 12 weeks of therapy (p = 0.016) showed an independent significant impact on overall survival in a multivariate analysis (Fig. 3). In cases where only 1–3 cycles were completed during therapy the mortality risk increased by the factor 9.9 (Exp(B) = 9.897; 95% HR 4.015–24.394). The opposite effect was associated with Abiraterone intake, which decreased the mortality risk by 68% (Exp(B) = 0.316; 95% HR 0.129–0.773).

Table 3
Cox-Regression (univariate)

Cox-Regression univariate	B	p-Value	Exp(B)	95% HR	
				Lower limit	Upper limit
Cycle 1–3 vs. 4–6	**2,028**	**< 0.001*** **	**7.597**	**4.153**	**13.895**
ADT/Hormone therapy		**0.003****			
Abiraterone (1) vs. 2/3	**-0.912**	**0.004****	**0.402**	**0.215**	**0.752**
Bicalutamide (2) vs. 1/3	**-1,144**	**0.002****	**0.319**	**0.155**	**0.653**
Enzalutamide (3) vs. 1/2	**-0.800**	**0.014***	**0.449**	**0.238**	**0.850**
BM-Distribution pattern		0.212			
I vs. II-IV	**-2,230**	**0.039***	**0.108**	**0.013**	**0.893**
II vs. I, III, IV	-1,970	0.062	0.139	0.018	1,106
III vs. I, II, IV	-1,768	0.095	0.171	0.021	1,364
IV vs. I-III	-1,497	0.173	0.224	0.026	1,929
Chemotherapy no vs. yes	-0.113	0.645	0.893	0.552	1,446
Radiotherapy no vs. yes	-0.091	0.748	0.913	0.524	1,590
Age ≤ 65 vs. >65	-0.220	0.505	0.802	0.420	1,534
AP ≤ 129 vs. >129 U/l	**-0.872**	**< 0.001*** **	**0.418**	**0.257**	**0.681**
BSI ≤ 1 vs. >1	-0.832	0.117	0.435	0.154	1,230
UR Cut-off ≤ 8 vs. >8	0.140	0.599	1,151	0.682	1,941
PSA Nonresponder vs. Responder	1,050	0.005	2,857	1,384	5,899

UR-Uptake ratio, BM-Bone marrow, BSI-Bone Scan Index, ADT-Androgen deprivation therapy, AP-Alkaline phosphatase, PSA-Prostate specific antigen, p-Value: * <0,05; **<0,01; ***<0,005

BM status in relation to AP value, ADT and BSI

Subsequently we performed a classification in relation to the cut-off value 8. The AP base value led us to conclude a significant difference in relation to UR (p = 0.002) in total. An AP base value of ≤ 129 U/l in combination with UR ≤ 8 resulted in an increased life expectancy of 5.8 months (Fig. 4a). Patients with UR > 8 lived 11.7 months longer at an AP base value of ≤ 129 U/l (Fig. 4b). Regarding ADT there were no significant OS advantages between the UR-subgroups, which is probably also owing to the small number of cases. The automatically calculated BSI was not significant (p = 0.104).

Cox-regression In Relation To Therapeutic Outcome

The correlate of therapeutic success was defined as the number of survival months of below or over 14.0 months, this value being the median survival of the overall collective. The model for multivariate Cox-analysis considered the parameters UR, ADT and AP base value (Table 4). As the variables radiation and chemotherapy were tested to be non-significant in the univariate Cox regression analysis, we created the interactive variables Radiation*UR-SIR as well Chemotherapy*UR-SIR in order to test potential interaction effects of these two impact factors (reverse selection). The multivariate analysis deliberately excluded factors which only occurred later in the course (e. g. PSA response, number of cycles). The analysis identified UR (p = 0.027) as the only significant factor. The interaction variables were not shown to have any impact. By contrast, if UR increased by one unit, chances for therapeutic success rose by9.6%.

Table 4
Cox-Regression of therapy success

Cox-Regression	B	p-Value	Exp(B)	95% HR	
				Lower limit	Upper limit
UR	0.092	**0.027***	**1,096**	1,010	1,189
UR-Uptake ratio, p-Value:* <0,05					

Discussion

We performed pretherapeutic BMS in order to stratify patients with a higher vulnerability of BM prior to radionuclide therapy. The current study primarily focused on the establishment of a (semi-)quantitative evaluation of der BMS via ROI-technique for the assessment of BM as a prognostic factor for the incidence of hematological side effects and successful management of radium-223-dichloride-therapy. Munz et al. described a semi-quantitative method for assessment of BM via ROI technique as early as 1984 [16]. However, the BM of patients who had already undergone radiotherapy was classified as non-assessable. Our results, by contrast, indicate that even BM with limited functionality in the sacroiliac area represent a correlate for the hematopoietic System in patients with mCRPC. For clarification, we initially identified UR smaller or larger 8 as a predictive value. Compared to the age-adjusted norm values identified by Huić et al., the identified cut-off of 8 correlated with the group of 40–59 year-old patients, which was calculated as 7.5 in the study of Huić et al. [17]. On the other hand the patient collective of Huić et al. excluded patients with prostate cancer [17]. As might be expected, it was shown that patients with UR > 8 and thus a high BM reserve and intact hematopoietic stem cells are more likely to show unimpaired hematopoiesis as those with UR ≤ 8. The pretherapeutic Hb values in patients with mCRPC and UR > 8 were 11% higher than in those with UR ≤ 8 (p = 0.001). Similar results were shown for the number of NG and erythrocytes. In addition, malignant manifestations in the bones and the BM can lead to higher AP values, which can thus be used as tumor progression markers during radium-223-dichloride treatment [22]. Regarding this biochemical parameter we were able to observe that the AP baseline value

in the serum of participants with UR > 8 was significantly (42%) lower compared to the group of UR ≤ 8 (p = 0.030).

Intravenously injected antibodies (AB) against surface antigens of human granulocytes lead – apart from intravasal binding to circulating granulocytes – to a largely selective bone marrow labelling. 45–55% of labelled AB are bound to the BM [23]. In accordance with Ivancévic et al. we positively assessed the high "target-to-background-ratio" of the here employed MAB in combination with SIR-ROI-Technik [18]. Nonetheless, the accuracy of ROI in the thoracolumbar region of the spine is limited due to superpositions, especially in the area of the liver [18]. This problem also became evident during identification of the UR in relation to the sternum, with the result that this method turned out to be infeasible in the further course. Moreover, the collected data have shown that identification of UR via a tetragonal ROI is simple and quick. The current results show a high reproducibility requiring only little time in the clinical routine. Blebea et al. 2007 published a concept for quantification of BM distribution by means of a combination of MRI and FDG-PET. The method is based on a quantitative SUV-determination on the hand and on MRI sequence analysis. Hence, we were able to gain insights on the structure and function of BM. The work group calculated the product of the overall vertebral activity and the BM volume [15]. At only 5 included patients, the validity of the latter study limited and in addition the two above-mentioned investigations are more cost-intensive and especially more time-consuming. The lower specificity of MRI in relation to malignant disease of the BM reduce the validity and the therapeutic benefit. As early as in 1997, Altehoefer et al. pointed out that MRI scan in cases of BM hyperplasia can lead to false-positive results [24]. In patients who have previously undergone radiation therapy, potential - although rare - BM hyperplasia should be considered as a possible source of false-positive BMS results [24].

For assessment of the prognostic value of radium-223-dichloride therapy it is important not only to consider myelotoxicity but also the survival advantage. In comparison to the ALSYMPCA-study with 14.9 months OS our study showed a median OS of 14.0 months, i.e. shortened by 0.9 months OS. This discrepancy may be owed to a progressive disease stadium in our collective. As expected, there was a positive prognostic impact regarding a physiological BM distribution pattern. Moreover, rising UR values increased the chance of reaching the median OS of the overall collective. Other studies with multivariate analysis demonstrated a significant impact of prior chemotherapy (p = 0.027), < 5 bone metastases (p = 0.014) and baseline AP < 115 U/l (p = 0.0013) [25]. These predictive factors cannot be confirmed by our data.

Finally, we would like to take a closer look at the impact of ADT. In the AFFIRM study the survival advantage with Enzalutamide after chemotherapy was 4.8 months [26]. Enzalutamide equally increased the survival advantage in our study (p = 0.014), while it was lower than Bicalutamide (p = 0.002) and Abiraterone (p = 0.004). Bicalutamide, yielded a median survival advantage of 5.4 months in chemotherapy-naïve patients as shown in the study by Dijkstra et al. [27]. In the TERRAIN-study fatigue and pain symptoms were less prevalent in Bicalutamide patients than in the Enzalutamide collective [28]. [illegible]ide may therefore be seen as the primary

treatment of choice based on the results of these studies. When considering progression-free survival, however, therapy with Enzalutamide would be preferable according to the STRIVE and TERRAIN studies reporting a median survival of 19.4 resp. 15.7 months in comparison to Bicalutamide with 5.7 vs. 5.8 months [28, 29]. Based on published results of the ERA-223-study the current recommendation is to apply radium-223-dichloride after two previous systemic therapies and evident progress only as a monotherapy as adverse effects were observed in combination with Abirateronacetate/Prednisolon [30]. According to the ERA-223-study (NCT020243678) the number of deaths rose by 34.7% in the course of a combined Abirateronacetate/radium-223-dichloride therapy compared to 28.2% in the placebo collective [31]. It is not possible to make comparisons with the ALSYMPCA study, as the application of modern hormone therapies like Abirateronacetate or Enzalutamide was entirely missing, this being one of the chief limitations of this study. Results on combined Enzalutamide/radium-223-dichloride studies (EnzaRadiCate; NCT02507570. PEACE III; NCT0219482) are largely pending. The current recommendation of CHMP (Committee for Medicinal Products for Human Use) is not to apply a combination of radium-223-dichloride and Enzalutamide. We would like to emphasize that according to our results all ADT preparations tended to show a positive impact on OS in the Cox regression analysis. Due to the small number of cases we can only infer a tendency at present. Further investigations should elaborate on the role of ADT.

Conclusion

A BM reserve in the amount of UR > 8 was associated with particularly low hematotoxicity of radium-223-dichloride-therapy. OS is not dependent on EBRT and/or prior chemotherapy at UR > 8, in other words a sufficient BM reserve is a positive predictive factor. The prognostic impact factors UR ($p = 0.027$) and BM distribution patterns in Group I ($p = 0.039$) were confirmed with statistical significance in this study. The impact of ADT needs to be clarified in current studies with larger case number and Placebo groups. It should be emphasized that a chief success of this study was to determine a prognostic threshold for quantification and, moreover, to generate a BM distribution algorithm. Pretherapeutic BMS can help to select patients, with a view to alleviating adverse effects and in order to enable application of all 6 radium-223-dichloride cycles. This method can make a useful contribution to therapy planning and this therapy can possibly serve as basis for other radionuclide therapies like PSMA-therapy and can be a starting point for further studies.

List Of Abbreviations

AB Antibodies

ADT Androgen deprivation therapy

AP Alkaline phosphatase

AUC Area under the curve

BM Bone marrow

BMS Bone Marrow Scintigraphy

BSI Bone Scan Index

CHMP Committee for Medicinal Products for Human Use

CI Confidential Interval

EBRT External percutaneous radiation therapy

HAMAs Human anti-mouse antibodies

Hb Hemoglobin

ICC Intra-correlation coefficient

LDH Lactate dehydrogenase

M Median

MAB Tc-99m-labelled monoclonal antibodies

mCRPC Metastasized castration resistant prostate cancer

MRI Magnetic resonance imaging

NG Neutrophil granulocytes

OS Overall survival

PSA Prostate specific antigen

ROC Receiver-Operating-Characteristics

ROI Regions of interest

SIR Sacroiliac region

UR Uptake ratio

<

Declarations

Funding: None.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of the Medical University of University Hospital Schleswig-Hosltein, Kiel (No. D575/18).

Informed consent: Informed consent was obtained from all individual participants included in the study.

Competing interests: The authors declare that they have no competing interests.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contribution statements: Wiebke Schüler: Data analysis, Manuscript editing. Marlies Marx: Statistical analysis, Manuscript editing. Maaz Zuharya: Protocol development. Michael Jüptner: Acquisition of data. Joshua-Kumaran Ranjan: Acquisition of data. Yi Zhao: Statistical analysis. Carsten Maik Naumann: contributed to conception. Ulf Lützen: Protocol development, Manuscript writing. All authors read and approved the final manuscript.

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Figures

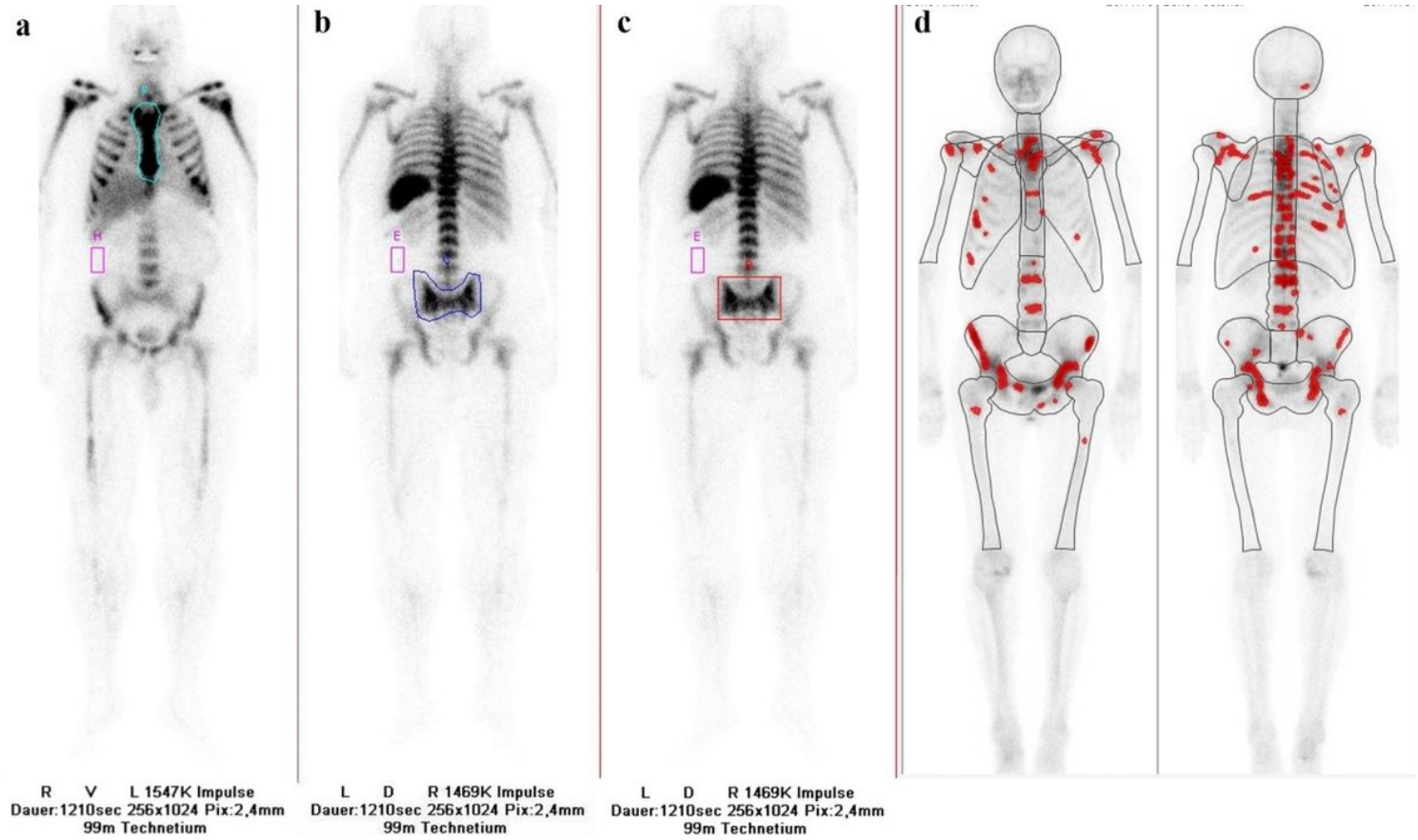

Figure 1

(a) Bonemarrowscan in the anterior viewwiththeregionofinterestplacedoverthe sternal area and thebackgroundregionbetweentherightKidney, spine and pelvis, (b) Bonemarrowscan in theposteriorviewwiththeirregularregionofinterestplacedoverthesacroiliacarea and thebackgroundregionbetweentheleftKidney, spine and pelvis, (c) Bonemarrowscan in theposteriorviewwiththerectangularregionofinterestplacedoverthesacroiliacarea and thebackgroundregionbetweentheleftKidney, spine and pelvis. (d) GraphicillustrationoftheBone Scan Index in the anterior and posteriorview. The redspotssupposedtobemetastaticbonemanifestations.

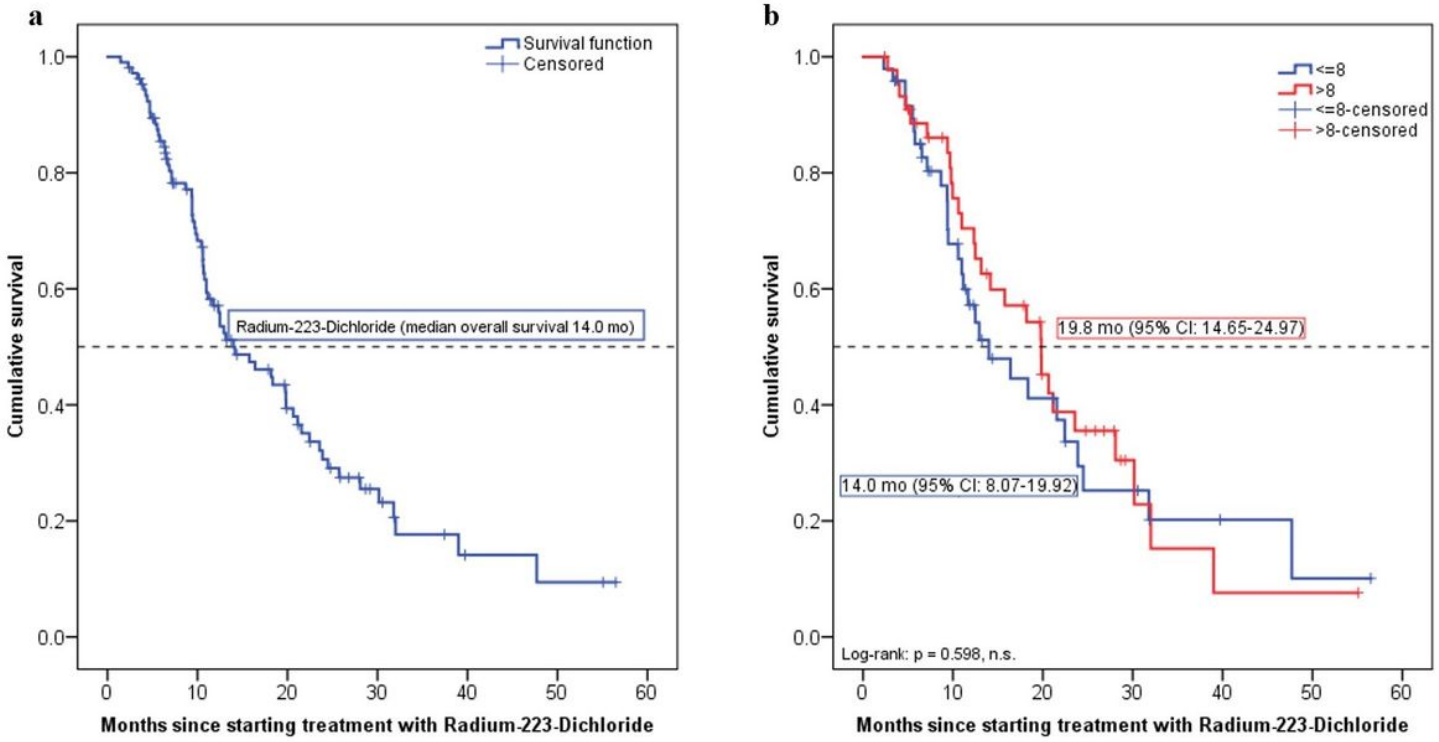

Figure 2

a/b Kaplan-Meier estimatesofoverallsurvival (OS) of all patients and OS by UR ≤/> 8

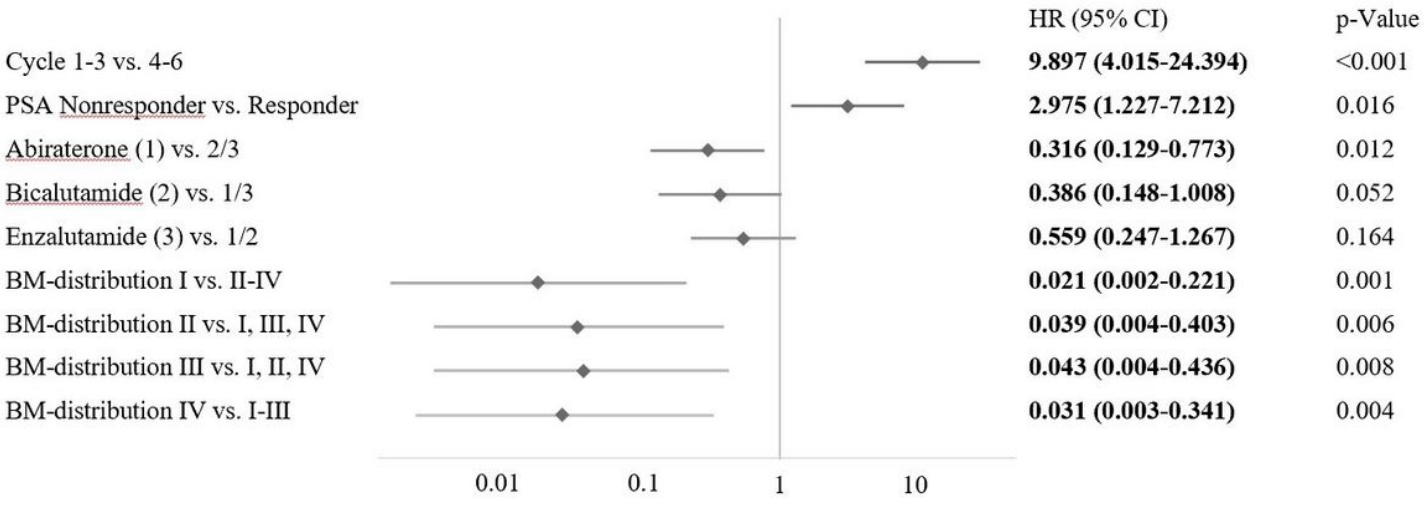

Figure 3

Forest-plot of Cox multivariate regressionanalysisof Overall survival, UR-Uptakeratio, PSA-Prostatespecificantigen

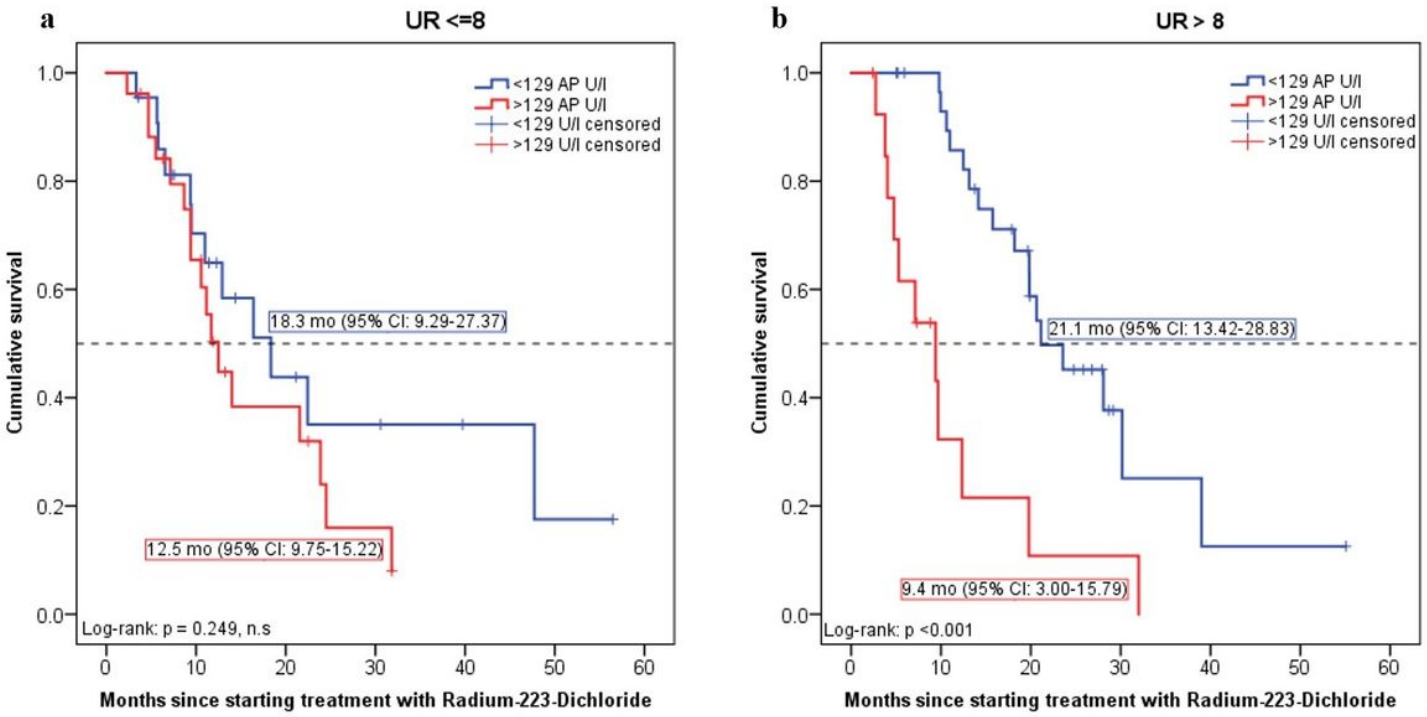

Figure 4

a/b Kaplan-Meier estimatesofoverallsurvivalbystratification in twogroups (UR≤ 8 and >8), a) cumulativesurvivalstratifiedbybaseline alkaline phosphataseconcentration (≤ 129 or>129 U/l) accordingto an UR≤ 8, b) cumulativesurvivalstratifiedbybaseline alkaline phosphataseconcentration (≤ 129 or>129 U/l) accordingto an UR>8