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Soluble B7-H4 and its Association with Clinical Characteristics and Prognosis in Patients with Early Breast Cancer

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Abstract

Background: Immunotherapy is a promising area for treatment of breast cancer (BC) that has transformed patient care. B7-H4 is highly expressed in BC and its expression correlates with the progression of the disease and a poor prognosis. B7-H4 is a potentially novel target for cancer therapy. The aim of this study was to explore whether its soluble form (sB7-H4) is a reliable marker for BC prognosis.

Methods: Using ELISA, we analyzed sB7-H4 in blood serum in a total of 572 early BC patients before the onset of therapy, in the neo-adjuvant setting (cohort 1, n=109) and in the adjuvant setting (cohort 2, n=463). In cohort 1, measurements were also performed after neo-adjuvant therapy (NACT).

Results: In cohort 1, sB7-H4 was delectable in blood serum in 27/109 (26%) patients before and in 50/109 (48%) patients after NACT. No significant differences between sB7-H4 levels before and after NACT were observed. No significant chances in sB7-H4 blood serum concentration levels before and after NACT were associated with clinical parameters, particular intrinsic subtypes or prognosis and the risk of recurrence. In cohort 2, the detection rate was 4% (18/461 patients). The median blood serum concentration levels in cohort 2 were significantly higher than in cohort 1 after NACT (p=0.04).

Conclusions: sB7-H4 concentration levels in serum of non-metastatic BC patients are neither associated with prognosis nor with clinical characteristics in the adjuvant and neo-adjuvant setting.

Keywords: sB7-H4, Breast cancer, Immune checkpoints

Abbreviations: ACT: Adjuvant Chemotherapy; BC: Breast Cancer; DFS: Disease-Free Survival; ER: Estrogen-Receptor; HER2: Human Epitdermal Growth Factor Receptor-2; IQR: Interquartile Range; NACT: Neoadjuvant Chemotherapy; OS: Overall Survival; pCR: Pathological Complete Response; PD-L1: Programmed Cell Death Ligand 1; pPR: Pathological Partial Response; PR: Progesterone-Receptor; ROC: Receiver Operating Curve; TNBC: Triple Negative Breast Cancer

Background

The development of cancer is associated with the growth of a suppressive tumor microenvironment including mechanisms of avoiding immune destruction by alternating the immune checkpoint pathway. Immune checkpoint blockades have shown dramatic effects in various tumor types including breast cancer (BC) [1]. However, this effect

has especially been observed in the triple negative BC (TNBC) subgroup, but also in a small subgroup of Luminal B and HER2-positive BC patients [2,3]. One possible explanation is higher tumor mutational load of TNBC and metastatic BC versus HER2-positive or luminal tumors and early BC, although the expression of immune-checkpoints in all subtypes of BC was identified [4,5]. Therefore, a better understanding of the interactions between immune cells and BC is needed to develop new therapeutic options and identify subgroups of patients that could benefit from the targeted therapies.

B7 family members have both, stimulatory and inhibitory effects on T cells and play a critical role in maintaining immune tolerance. They can help a tumor to escape from host surveillance [6]. B7-H4 is a co-stimulatory ligand that inhibits T-cell responses by interacting with as yet unidentified receptors [7]. B7-H4 is highly expressed in various types of neoplasms including ovarian, endometrial or BC and its expression correlates with the progression of the disease and a poor prognosis in many cases [8-10]. Moreover, B7-H4 is a potentially novel target for cancer therapy [11]. B7-H4 has a membrane-bound and soluble form (sB7-H4), which has also been detected in serum of patients with cancer with its expression closely related to progression and prognosis [12]. However, it remains unclear whether sB7-H4 is associated with diagnostics and outcomes in BC patients.

Immune checkpoint inhibitors are only effective in a subset of patients, and the identification of biomarkers that predict response to therapy is crucial to increase the rates of responders. Moreover, soluble serum biomarkers are useful diagnostic tools because they can reflect the tumor status and predict patient prognosis. Here, we investigate the distribution of blood serum sB7-H4 in patients with early BC across different clinico-pathological characteristics and the prognostic value sB7-H4 for patients' outcome. The aim of this study was to explore whether sB7-H4 is a reliable marker for BC prognosis.

Methods

Patient population and patient characteristics

In total, 572 primary, non-metastatic BC patients, diagnosed between 2004 and 2009 at the University Hospital of Essen, Department of Gynecology, were analyzed. Cohort 1 consisted of 109 pts in the neo-adjuvant setting and cohort 2 of 463 pts in the adjuvant setting. In both groups, serum was obtained before surgery and in cohort 1, additionally after neo-adjuvant chemotherapy (NACT).

The eligibility criteria were: histologically proven BC, no severe uncontrolled comorbidities or medical conditions and no further malignancies at the time of enrollment or in the patient history, completion of neoadjuvant or adjuvant treatment according to current national guidelines [13] including adjuvant chemotherapy (ACT) and NACT (anthracyclines, taxanes, cyclophosphamide, carboplatin, gemcitabine, 5-fluorouracil), anti-hormonal therapy in the case of hormone responsive tumors (tamoxifen or an aromatase inhibitor), Herceptin (after FDA approval in November 2006) in the case of HER2 positivity and radiotherapy. For each of the 572 patients, the tumor type, TNM-staging and grading were assessed in the Institute of Pathology, at the University Hospital Essen as part of the West German Comprehensive Cancer Center. In the neoadjuvant setting, pathological response to therapy was defined according to the grading system of Sinn and colleagues [Sinn=0, no pathological response; Sinn=1-3, pathological partial response (pPR) and Sinn=4, pathological complete response (pCR) [14].

Patients positive for disseminated tumor cells in the bone marrow were recommended to complete a prescription of clodronate (2 × 520 mg/d) for at least two years.

Sampling of Serum and Measurement of sB7-H4 protein levels

9 ml of blood were collected from each patient using S-Monovettes (Sarstedt AG & Co, Nümbrecht, Germany), stored at 4°C and processed within 4 hours to avoid blood cell lysis. Blood fractionation was carried out by centrifugation for 10 min at 2500 × g. Subsequently, 3 – 4 ml of the upper phase, constituting blood serum, were removed and stored at -80°C. All samples were assayed in batch form for blood serum protein levels of sB7-H4. sB7-H4 serum levels were analyzed using the sandwich Enzyme-Linked Immunosorbent Assay Kit (Cusabio, Cologne, Germany) according to the manual instructions. For ELISA measurement, 100 µl undiluted serum samples and control samples were dispensed into wells coated with an antibody specific for B7-H4 and incubated for 2 hours at 37°C. Subsequently, after removing any unbound substances, a biotin-conjugated antibody specific for B7-H4 was added to the wells for 1 hour at 37°C. After washing three times, 100 µL of avidin conjugated Horseradish Peroxidase (HRP) was added for 1 h at 37°C. Subsequently, after washing five times, 90 µL of substrate solution containing TMB (Tetramethylbenzidine) was added for 15-30 minutes at 37°C, protected from light. Color development was stopped by the addition of 50 µl stop solution to each well and the degree of enzymatic turnover of the substrate was

investigated by dual-wavelength absorbance measurement at 450 and 620 nm as a reference wavelength within 5 minutes using an ELISA reader (TECAN, Model Sunrise; Austria GmbH, Grodig, Austria) and the data analysis software Magellan™ (TECAN, Mannedorf, Switzerland). To quantify blood serum concentration levels of B7-H4, a non-linear regression model (4-parameter Marquardt) was used with a log/lin type of graph according to the manufacturer's instructions. The observed absorbance was directly proportional to the concentration level of sB7-H4 in the samples, which was calculated from the calibration curve. The sB7-H4 serum levels were expressed in ng/mL according to the established standard curve (detection range: 7.8-500 ng/mL). The minimum detectable dose of sB7-H4 was typically less than 1.95 ng/mL. The lower limit of detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. Intra-assay variation was <8%, while inter-assay variation was <10%.

Statistical analysis

The distribution of sB7-H4 in each study group was different from normal. Descriptive statistics were computed and reported as median with interquartile range (IQR) or frequency counts (%). The differences between two groups were defined by Mann-Whitney U test or paired Wilcoxon test. More than two groups were analyzed using Kruskal-Willis test. Differences in frequency counts were analyzed using the chi-squared test. Receiver Operating Curve (ROC) curve analysis was performed to obtain cut-off values representing the optimal separation of survival curve. The optimal cut-off value, i.e., the threshold that maximizes the sum of (sensitivity + specificity) was calculated according to Youden [15]. Kaplan-Meier analysis was performed to analyze progression free survival (PFS) and overall survival (OS) probabilities. The difference between survival curves was assessed by using the log rank test. Age-adjusted hazard ratios (HR) with corresponding 95%-confidence intervals (95%-CI) were calculated by using Cox proportional hazards regression. All analyses were performed using the MedCalc version 17.9.7 (MedCalc Software bvba, Ostend, Belgium) and the R statistical package version 3.4.0.

Results

The clinical characteristics of all patients are shown in Tables 1 and 2. The median age was 51 years (IQR 43,5-61) in cohort 1 and 61 years (IQR 51-68) in cohort 2. The predominant histological subtype was invasive ductal carcinoma in both groups (72% and 76%). In cohort 1, the majority of patients (58%) were pre- and perimenopausal whereas most of the patients in cohort 2 were postmenopausal (76%). In contrast to cohort 2 with most of the patients characterized as T1 (65%), no lymph node involvement (68%) and grade 2 (54%) tumors, 69% of the patients in cohort 1 showed a T2 tumor, 52% of the patients were node positive and had more aggressive tumors, characterized as grade 2 (44%) and grade 3 (48%). When stratifying according to BC subtypes, 17% were triple-negative and 28% Her2-positive in cohort 1. In cohort 2, the values were 11% and 12% while all other patients (77%) were characterized as hormone receptor-positive.

In cohort 1, 102/109 patients (93.58%) received NACT and 7 patients (6.42%) a neo-adjuvant endocrine therapy. Overall, response to therapy resulted in a ratio of 94% (22% pCR; 73% pPR) of responders and 6% of non-responders. In cohort 2, 220/463 (48%) of the patients received ACT, 168/463 (36%) did not get chemotherapy and in 75/463 patients (16%), no information was available.

In cohort 1, sB7-H4 blood serum concentration was delectable in 27/109 (26%) patients before and in 50/109 (48%) patients after NACT. In cohort 2, sB7-H4 blood serum concentration was only delectable in 18/461 (4%) patients.

Comparison of sB7-H4 in blood serum between groups

The median blood serum concentration levels in cohort 1 were 10.49 ng/ml (IQR 7.78-17.27) before and 8.43 ng/ml (IQR 3.46-18.79) after NACT. For cohort 2, the value was 16.96 ng/ml (IQR 6.49-30.59) which was significantly higher than in cohort 1 after NACT (p=0.04) but not before NACT. No significant differences between patients before and after NACT were observed (Figure 1).

sB7-H4 serum levels among particular intrinsic subtypes of BC in cohort 1

Median sB7-H4 blood serum concentration levels in cohort 1 before NACT was 13.5 ng/ml (IQR 8.8-24.2) for the ER+ PR+ HER2- subtype, 9.13 ng/ml (IQR 6.84-13.26) for the HER2+ subtype and 8.92 ng/ml (IQR 6.84-14.87) for the TNBC subtype, respectively. After NACT, the values were 8.26 ng/ml (IQR 3.71-16.48), 8.37 ng/ml (IQR 3.34-18.97) and 9.71 ng/ml (IQR 2.01-26.29), respectively. However, differences before (p=0.25) and after NACT(p=0.96) were not statistically significant (Figure 2).

In cohort 2, the comparison among intrinsic subtypes was not performed due to low detection rates of sB7-H4 in blood serum.

Table 1: Clinical data of patients in the neoadjuvant cohort (cohort I).

	Total (%) Neoadjuvant cohorte (% of all applicable/known)	**p-value**
Total	109	
Menopausal Status		
Pre- and perimenopausal	64/109 (58.72)	0.06
Postmenopausal	45/109 (41.28)	
Histology		
Ductal	73/102 (71.57)	<0.0001
Lobular	16/102 (15.69)	
Others	13/102 (12.75)	
nk	7/109 (6.42)	
Grading		
I	8/108 (7.41)	<0.0001
II	48/108 (44.44)	
III	52/108 (48.15)	
nk	1/109 (0.92)	
Tumor before NACT (cT)		
T1	17/109 (15.60)	<0.0001
T2	75/109 (68.81)	
T3	11/109 (10.09)	
T4	6/109 (5.50)	
Nodal status before NACT (cN)		
Node negative	53/109 (48.62)	<0.0001
Node positive	56/109 (51.38)	
N1	47/109 (43.12)	
N2	8/109 (7.23)	
N3	1/109 (0.92)	
Pathological response		
Response	101/107 (94,39)	<0.0001
Complete response	23/107 (21.50)	
Partial response	78/107 (72.90)	
No response	6/107(5.61)	
nk	2/109 (1.83)	
Neoadjuvant therapy		
Chemotherapy	102/109 (93.58)	<0.0001
Endocrine therapy	7/109 (6.42)	
Immunhistochemical Subtype		
ER-, PR-, HER2-	18/109 (16.51)	<0.0001
HER2+	13/109 (27.52)	
(ER+ and/ or PR+, HER2-)	61/109 (55.96)	
Recurrence		
No	72/103 (69.90)	<0.0001
Yes	31/103 (30.20)	
nk	6/109 (5.50)	
Distant Recurrence		
No	78/102 (76.47)	<0.0001
Yes	17/102 (16.67)	
nk	7/109 (6.42)	

ER = estrogen receptor, PR = progesterone receptor, HER2 = human epidermal growth factor receptor-2, TNBC = triple-negative breast cancer, nk = not known; nd = not done

Table 2: Clinical data of patients in the adjuvant cohort (cohort II).

	Total (% of all applicable/known)	**p-value**
Total	463	
Menopausal Status		
Pre- and perimenopausal	109/463 (23.54)	<0.0001
Postmenopausal	354 (76.46)	
Histology		
Ductal	354/453 (78.16)	<0.0001
Lobular	60/453 (13.25)	
Others	39/453 (8.61)	
nk	10/463 (2.16)	
Grading		
I	90/463(19.44)	<0.0001
II	251/463 (54.21)	
III	122/463 (26.35)	
Tumor size at first diagnosis (pT)		
T1	300/460 (65.22)	<0.0001
T2	140/460 (30.43)	
T3	14/460 (3.04)	
T4	6/460 (1.30)	
nk	3/463 (0.65)	
Nodal Status at first diagnosis		
Node negative	313/462 (67.75)	<0.0001
Node positive	149/462 (32.25)	
N1	136/462 (29.44)	
N2	10/462 (2.16)	
N3	3/462 (0.65)	
nk	1 (0.22)	
Chemotherapy		
No	168/388 (43.30)	<0.0001
Yes (adjuvant)	220/388(56.70)	
nk	75/463(16.20)	
Immunhistochemical Subtype		
ER-, PR-, HER2-	49/459 (10.68)	<0.0001
HER2+	55/459 (11.73)	
ER+ and/ or PR+, HER2-	355/459 (77.34)	
nk	4/463(0.86)	
Recurrence		
No	360/384 (93,75)	<0.0001
Yes	24/384 (6.35)	
nk	79/463 (17.06)	
Distant Recurrence		
No	266/283 (94.00)	<0.0001
Yes	17/283 (16.01)	
nk	180/463 (38.88)	

ER = estrogen receptor, PR = progesterone receptor, HER2 = human epidermal growth factor receptor-2, TNBC = triple-negative breast cancer, nk = not known; nd = not done

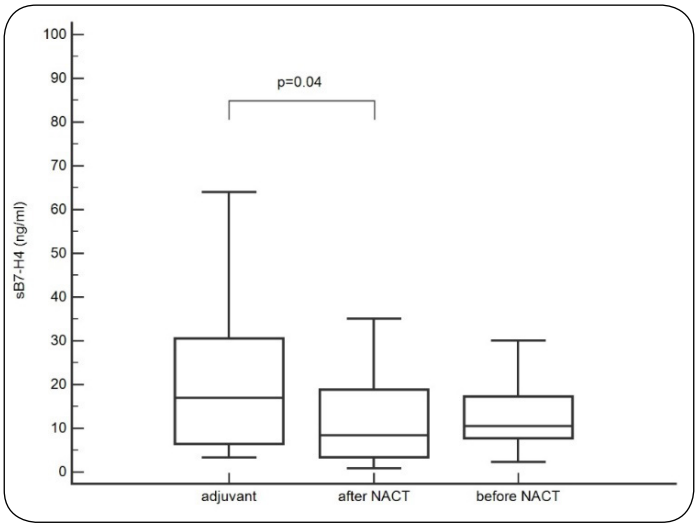


Figure 1: Comparision of sB7-H4 levels in blood serum between cohort 1 (n=109) before (10.49 ng/ml; IQR 7.78-17.27) and after NACT (8.43 ng/ml; IQR 3.46-18.79) versus cohort 2 (16.96 ng/ml; IQR 6.49-30.59, n=463).

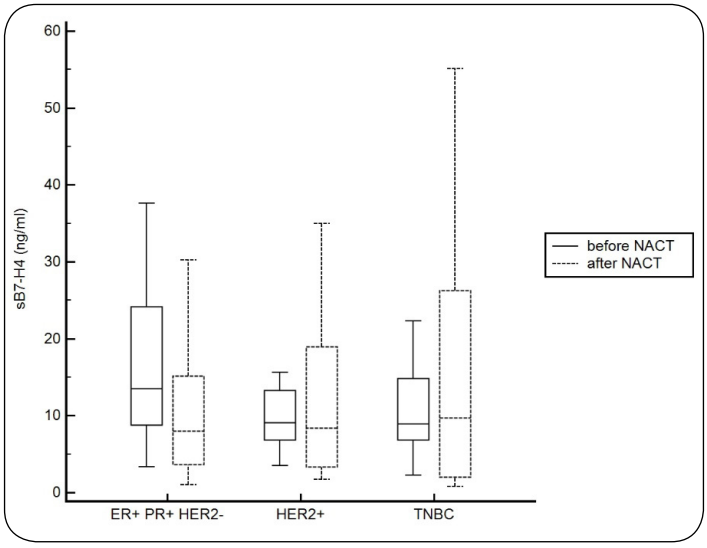


Figure 2: Box plots for sB7-H4 blood serum concentration levels among particular intrinsic subtypes of BC in cohort 1 (N=109).

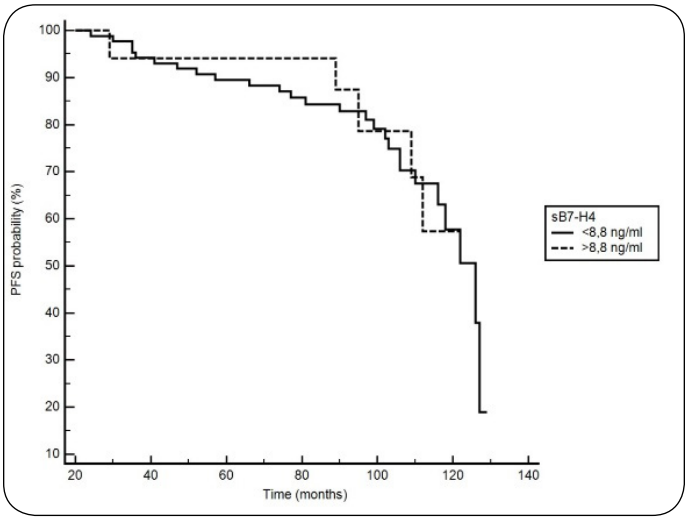
Association of sB7-H4 and clinicopathological characteristics in cohort 1

No significant associations between clinical characteristics and chances in sB7-H4 blood serum concentration levels before and after NACT were obtained (Table 3).

The prognostic value of sB7-H4 in blood serum

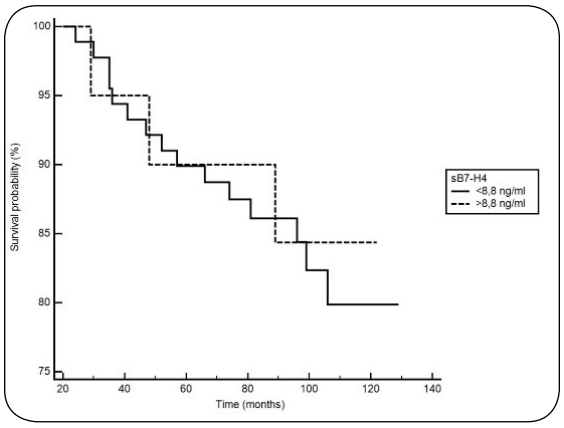
To obtain cut-off values representing the optimal separation of survival curve, Receiver Operating Curve (ROC) analysis was performed. For patients before NACT, the area under the curve (AUC) was 0.68 (95%-CI 0.47-0.84) with the optimal threshold of 8.8 ng/mL for OS and 0.61 (95%-CI 0.39-0.8) for DFS with the optimal threshold of 8.8 ng/mL. For patients after NACT, the AUC was 0.63 (95%-CI 0.48-0.76 with the optimal threshold of 3.6 ng/mL for OS) and 0.53 (95%-CI 0.38-0.68 with the optimal threshold of 16.3 ng/mL for DFS. The survival analysis showed no significant differences in OS and in DFS before and after NACT (Figure 3 A) DFS in patients before NACT, B) OS in patients before NACT, C) DFS in patients after NACT, D) OS in patients after NACT).

A) Kaplan–Meier plots of disease-free survival in patients before NACT



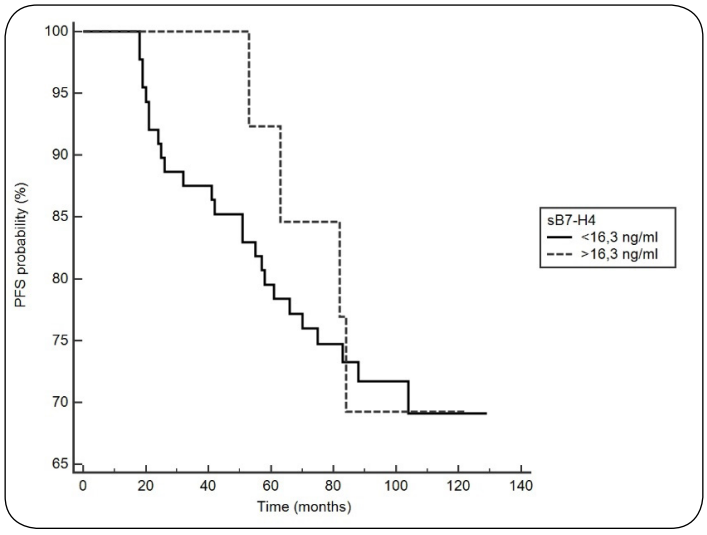
Logrank p = 0.26

B) Kaplan–Meier plots of overall survival in patients before NACT



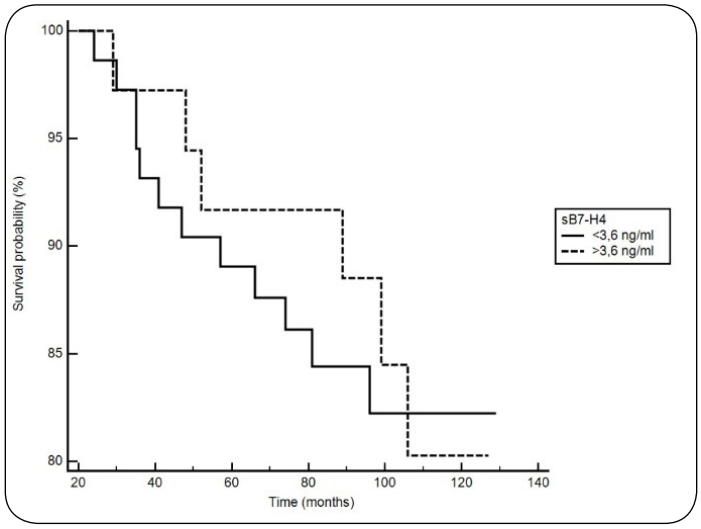
Logrank p = 0.36

C) Kaplan–Meier plots of disease-free survival in patients after NACT



Logrank p = 0.89

D) Kaplan–Meier plots of overall survival in patients after NACT



Logrank p = 0.46

Figure 3: Overall survival and progression-free survival of patients from cohort 1 stratified by sB7-H4 detection in blood serum. A) DFS in patients before NACT, B) OS in patients before NACT, C) DFS in patients after NACT, D) OS in patients after NACT).

Age-adjusted Cox regression analysis indicated that sB7-H4 levels before and after NACT did not influence the prognosis and risk of recurrence (Table 4).

Discussion

B7-H4 plays a significant role in tumor escape from the immune surveillance [16]. While the mechanism is still not clear, sB7-H4 may also play a role in cancer development through negative regulation of T-cell immunity. So far, there is no evidence in the literature comparing sB7-H4 blood serum levels with clinical characteristics and outcomes of patients with early BC. Here, we assessed changes in sB7-H4 blood serum levels in paired pre-NACT and post-NACT as well as adjuvant serum samples and correlated these findings with clinico-pathological parameters and prognosis. Although we observed higher sB7-H4 levels after therapy, there were no significant associations between sB7-H4 and prognosis in the neo-adjuvant setting. In addition, no differences in sB7-H4 levels could be documented for the different BC subtypes.

Expression of B7-H4 in tumor tissue has been linked to a worse prognosis in some types of cancer [17]. However, data in the context of BC are inconsistent. Huang et al. showed that the OS rate of patients with higher B7-H4 expression was significantly worse than in those with lower expression [18]. Similarly, TNBC patients with B7-H4 overexpression had significantly shorter survival and recurrence time than those with low B7-H4 expression [19]. These data suggest that B7-H4 might be a potential negative prognostic indicator.

In contrast, other studies showed that B7-H4 expression was not associated with worse survival in BC [20], and expression of B7-H4 has even been linked to a favorable 5-year PFS [21]. There is no data available with regard to the prognostic features of sB7-H4 in BC patients. We found that sB7-H4 was not associated with prognosis, however, due to the limited data in some BC subgroups, we could not perform subgroup analyses for survival in the adjuvant setting. Nevertheless, we speculate that sB7-H4 may be a part of tumor-immune tolerance because B7-H4 is a negative regulator of immune response which might be important for BC patients. Treatment with antibodies targeting B7-H4 may result in a reduction of tumor progression and better patient outcomes. Indeed, an in vivo study using humanized animal model showed that a B7-H4/CD3-bispecific antibody might be a therapeutic agent against B7-H4-expressing tumors [11]. Therefore, it is essential to identify biomarkers that can predict the possible response to anti-B7-H4 treatment. The existence of sB7-H4 could be a predictive marker for immunotherapy targeting T lymphocytes as suggested by Ohki et al. [22].

B7-H4 is frequently expressed on tumor cells including BC. Data on the expression among particular intrinsic subtypes of BC— defined by expression of ER, PR or HER2—is inconsistent [20,21]. In our study, sB7-H4 was

Table 3: Changes in sB7-H4 blood serum concentration levels in patient cohort 1.

Parameter	sB7-H4 (ng/ml) Before NACT; median (IQR)	n	P value	sB7-H4 (ng/ml) After NACT; median (IQR)	n	P value
Menopausal status						
Pre-menopausal	9.03 (7.32-13.32)	16	0.18	9.80 (3.34-16.43)	26	0.85
Post-menopausal	13.5 (9.37-23.70)	11		7.98 (3.53-20.55)	24	
Histology						
Ductal	12.14 (7.98-16.72)	20	0.49	11.02 (3.64-19.16)	35	0.17
Lobular	8.80 (4.82-18.16)	3		13.69 (8.43-39.73)	4	
Others	20.25 (10.49-30.01)	2		3,34 (1,7-10,29)	5	
Tumor size						
cT1	8.93 (6.41-14.81)	7	0.42	10.99 (5.33-14.65)	6	0.28
cT2	10.39 (7.98 – 16.28)	16		6.15 (3.34-18.79)	38	
<cT2	17.03 (10.78-25.64)	4		22.11 (8.59-30.97)	6	
Nodal status						
cN0	12.95 (7.98-18.45)	12	0.8	5.33 (2.91-12.13)	19	0.8
cN1	10.39 (6.19-17.82)	14		12.27 (3.53-21.54)	27	
<cN1	8.8	1		8.27 (4.57-28.48)	4	
Tumor Grading						
G1	21.73 (13.31-31.63)	4	0.19	10.96 (3.45-37.57)	8	0.81
G2	9.61 (6.62-18.45)	12		11.02 (4.63-14.59)	19	
G3	10.49 (7.78-13.32)	11		6.09 (3.37-19.16)	23	
Distant metastasis						
No	9.81 (6.62-16.72)	20	0.92	8.42 (3.75-19.23)	30	0.37
Yes	8.92 (8.84-11,81)	3		5.81 (3.34-12.27)	14	
Tumor subtyp						
ER+ PR+ Her2-	13.5 (8.81-24.21)	13	0.25	8.27 (3.71-16.45)	29	0.93
Her2+	9.13 (6.84-13.27)	9		5.33 (3.26-17.07)	13	
TNBC	8.93 (6.84-14.88)	5		10,99 (2,46-32,96)	8	
Response to the therapy						
Complete remission	7.58 (6.12-15.23)	5	0.71	4.23 (3.10-12.35)	11	0.41
Partial remission	11.09 (8.58-16.72)	20		11.42 (3.50-19.16)	35	
No remission	10.49	1		4.98 (4.42-5.53)	2	
Recurrence						
No	9.13 (6.40-17.27)	19	0.45	8.42 (3.54-19.10)	28	0.72
Yes	10.49 (8.89-17.08)	5		6.09 (3.43-15.07)	17	

Table 4: Results from Cox regression analysis of patients in cohort 1.

sB7-H4 (ng/ml)	OS		DFS	
	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Cohort 1 before NACT	0.91 (0.73-1.12)	0.37	0.94 (0.85-1.04)	0.28
Cohort 1 after NACT	0.94 (0.84-1.05)	0.29	0.99 (0.96-1.03)	0.94
Cohort 2	1.00 (0.95-1.05)	0.98	1.02 (0.97-1.07)	0.29

independent of intrinsic BC subtypes. Since the source and function of sB7-H4 is not known, it is not clear whether serum sB7-H4 reflects the expression of B7-H4 in tumor tissue. Kamimura et al. [23] demonstrated that sB7-H4 is secreted in inflammatory environments and it was also reported to act as a decoy molecule that blocks suppressive functions of cell-associated B7-H4 leading to enhanced T-cell-mediated autoimmune responses [24]. Zhang et al. [25] suggested that different origins of sB7-H4 define its distinct structure and function. Nevertheless, there is a growing body of evidence indicating that sB7-H4 negatively regulates T-cells and plays a regulatory role in immune tolerance [26,27].

On the other hand, chemotherapy may have beneficial effects on anticancer immunity by enhancing mutational load or direct elimination of immunosuppressive cells [28-30]. This fact may explain the low rate of sB7-H4 positive patients in adjuvant setting. Thus, understanding the effect of DNA-damaging agents on the immune system is critical to identify optimal strategies that combine checkpoint inhibitors and chemotherapy agents. In our study, the detection of serum sB7-H4 almost doubled after NACT versus before NACT. Some preclinical studies have suggested that immune checkpoint expression like Programmed Cell Death Ligand 1 (PD-L1) might be stimulated by chemotherapy; others observed a significant decrease in PD-L1 expression after NACT in BC patients [31,32]. The changes collectively induced by NACT in immune-checkpoint expression could provide a rationale for the use of immune checkpoint inhibitors in the neoadjuvant setting for BC patients.

The primary limitation of this study is its retrospective character, the lack of corresponding B7-H4 tissue availability and its expression in the tumor microenvironment. However, our study performed on a representative group of BC patients suggests a lack of an association between sB7-H4 serum levels and prognosis. The effects of anti-B7-H4 therapy in BC patients are unknown. Since B7-H4 remains a candidate for targeted inhibition in cancer immunotherapy, further prospective studies with blockade of B7-H4 in association with sB7-H4 levels should be proposed to investigate the applicability to anti-B7-H4 immune therapy in BC.

Conclusion

sB7-H4 concentration levels in serum of non-metastatic BC patients are neither associated with prognosis nor with clinical characteristics in the adjuvant and neo-adjuvant setting.

Declarations

Ethics approval and consent to participate

All blood samples were obtained and collected after written informed consent from all subjects using protocols approved by the clinical Ethic committee of the University Hospital Essen (17-7495-BO).

Consent for publication

Not applicable.

Availability of data and material

The datasets for the current study are available from the corresponding author upon request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

PM gave substantial contributions to conception and design, acquisition, analysis and interpretation of data, and was a major contributor in writing the manuscript. AKB gave substantial contributions to conception, design, acquisition and interpretation of data, drafting and revising the article and final approval of the version to be published. SKB gave substantial contributions to conception and design, interpretation and revising the article and final approval of the version to be published. BS gave substantial contributions to analysis and interpretation of data, revising the article and final approval of the version to be published. OH and RK gave substantial contributions to acquisition of data, revising the article and final approval of the version to be published. All authors read and approved the final manuscript.

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References

1. Schmid P, Adams S, Rugo HS, et al. IMpassion130 Trial Investigators. Atezolizumab and Nab-paclitaxel in advanced triple-negative breast cancer. New Engl J Med. 2018; 379: 2108-2121. DOI: https://doi.org/10.1056/nejmoa1809615
2. Schmid P, Cortes J, Pusztai L, et al. KEYNOTE-522 Investigators. Pembrolizumab for Early Triple-Negative Breast Cancer. N Engl J Med. 2020; 382: 810-821. DOI: https://doi.org/10.1056/nejmoa1910549
3. Anurag M, Zhu M, Huang C, et al. Immune Checkpoint Profiles in Luminal B Breast Cancer (Alliance). J Natl Cancer Inst. 2020; 112: 737-746. DOI: https://doi.org/10.1093/jnci/djz213
4. Kurozumi S, Inoue K, Matsumoto H, et al. Clinicopathological values of PD-L1 expression in HER2-positive breast cancer. Sci Rep. 2019; 9: 16662. DOI: https://dx.doi.org/10.1038%2Fs41598-019-52944-6
5. Barroso-Sousa R, Jain E, Cohen O, et al. Prevalence and mutational determinants of high tumor mutation burden in breast cancer. Ann Oncol. 2020; 31: 387-394. DOI: https://doi.org/10.1016/j.annonc.2019.11.010
6. Chen L. Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. Nat Rev Immunol. 2004; 4: 336-347. DOI: https://doi.org/10.1038/nri1349
7. Yi KH, Chen L. Fine tuning the immune response through B7-H3 and B7-H4. Immunol Rev. 2009; 229: 145-151. DOI: https://dx.doi.org/10.1111%2Fj.1600-065X.2009.00768.x
8. Miyatake T, Tringler B, Liu W, et al. B7-H4 (DD-O110) is overexpressed in high risk uterine endometrioid adenocarcinomas and inversely correlated with tumor T-cell infiltration. Gynecol Oncol. 2007; 106: 119-127. DOI: https://doi.org/10.1016/j.ygyno.2007.03.039
9. Simon I, Zhuo S, Corral L, et al. B7-h4 is a novel membrane-bound protein and a candidate serum and tissue biomarker for ovarian cancer. Cancer Res. 2006; 66: 1570-1575. DOI: https://doi.org/10.1158/0008-5472.can-04-3550
10. Salceda S, Tang T, Kmet M, et al. The immunomodulatory protein B7-H4 is overexpressed in breast and ovarian cancers and promotes epithelial cell transformation. Exp Cell Res. 2005; 306: 128-141. DOI: https://doi.org/10.1016/j.yexcr.2005.01.018
11. Iizuka A, Nonomura C, Ashizawa T, Kondou R, Ohshima K, Sugino T, et al. A T-cell-engaging B7-H4/CD3-bispecific Fab-scFv Antibody Targets Human Breast Cancer. Clin Cancer Res. 2019; 25: 2925-2934. DOI: https://doi.org/10.1158/1078-0432.ccr-17-3123
12. Azuma T, Sato Y, Ohno T, Azuma M, Kume H. Serum soluble B7-H4 is a prognostic marker for patients with non-metastatic clear cell renal cell carcinoma. PLoS One. 2018; 13: e0199719. DOI: https://dx.doi.org/10.1371%2Fjournal.pone.0199719
13. AGO guidelines; https://www.ago-online.de/de/infothek-fuer-aerzte/ leitlinienempfehlungen/mamma
14. Sinn HP, Schmid H, Junkermann H, Huober J, Leppien G, Kaufmann M, et al. Histologic regression of breast cancer after NACT [in German]. Geburtshilfe Frauenheilkd. 1994; 54: 552-558. DOI: https://doi.org/10.1055/s-2007-1022338
15. Youden WJ. Index for rating diagnostic tests. Cancer. 1950; 3: 32□35. DOI: https://doi.org/10.1002/1097-0142(1950)3:1%3C32::aid-cncr2820030106%3E3.0.co;2-3
16. Liu WH, Chen YY, Zhu SX, Li YN, Xu YP, Wu XJ, et al. B7-H4 expression in bladder urothelial carcinoma and immune escape mechanisms. Oncol Lett. 2014; 8: 2527-2534. DOI: https://doi.org/10.3892/ol.2014.2522
17. Fauci JM, Straughn JM Jr, Ferrone S, Buchsbaum DJ. A review of B7-H3 and B7-H4 immune molecules and their role in ovarian cancer. Gynecol Oncol. 2012; 127: 420-425. DOI: https://doi.org/10.1016/j.ygyno.2012.08.017
18. Huang H, Li C, Ren G. Clinical significance of the B7-H4 as a novel prognostic marker in breast cancer. Gene. 2017; 623: 24-28. DOI: https://doi.org/10.1016/j.gene.2017.04.003
19. Wang L, Yang C, Liu XB, Wang L, Kang FB. B7-H4 overexpression contributes to poor prognosis and drug-resistance in triple-negative breast cancer. Cancer Cell Int. 2018; 18: 100. DOI: https://doi.org/10.1186/s12935-018-0597-9
20. Altan M, Kidwell KM, Pelekanou V, et al. Association of increased B7 protein expression by infiltrating immune cells with progression of gastric carcinogenesis. Medicine (Baltimore). 2019; 98: e14663. DOI: https://doi.org/10.1097/md.0000000000014663
21. Lee DW, Ryu HS, Jin MS, et al. Immune recurrence score using 7 immunoregulatory protein expressions can predict recurrence in stage I-III breast cancer patients. Br J Cancer. 2019; 121: 230-236. DOI: https://doi.org/10.1038/s41416-019-0511-9
22. Ohki S, Shibata M, Gonda K, et al. Circulating myeloid-derived suppressor cells are increased and correlate to immune suppression, inflammation

and hypoproteinemia in patients with cancer. Oncol Rep. 2012; 28: 453-458. DOI: https://doi.org/10.3892/or.2012.1812

23. Kamimura Y, Kobori H, Piao J, et al. Possible involvement of soluble B7-H4 in T cell-mediated inflammatory immune responses. Biochem Biophys Res Commun. 2009; 389: 349-353. DOI: https://doi.org/10.1016/j.bbrc.2009.08.144
24. Azuma T, Zhu G, Xu H, et al. Potential role of decoy B7-H4 in the pathogenesis of rheumatoid arthritis: a mouse model informed by clinical data. PLoS Med. 2009; 6: e1000166. DOI: https://doi.org/10.1371/journal.pmed.1000166
25. Zhang L, Wu H, Lu D, et al. The costimulatory molecule B7-H4 promote tumor progression and cell proliferation through translocating into nucleus. Oncogene. 2013; 32: 5347-5358. DOI: https://dx.doi.org/10.1038%2Fonc.2012.600
26. Leandersson P, Kalapotharakos G, Henic E, et al. A Biomarker Panel Increases the Diagnostic Performance for Epithelial Ovarian Cancer Type I and II in Young Women. Anticancer Res. 2016; 36: 957-965. Available from: https://pubmed.ncbi.nlm.nih.gov/26976984/
27. Thompson RH, Zang X, Lohse CM, et al. Serum-soluble B7x is elevated in renal cell carcinoma patients and is associated with advanced stage. Cancer Res. 2008; 68: 6054-6058. DOI: https://doi.org/10.1158/0008-5472.can-08-0869
28. Galluzzi L, Buque A, Kepp O, Zitvogel L, Kroemer G. Immunological effects of conventional chemotherapy and targeted anticancer agents. Cancer Cell. 2015; 28: 690-714. DOI: https://doi.org/10.1016/j.ccell.2015.10.012
29. Gotwals P, Cameron S, Cipolletta D, et al. Prospects for combining targeted and conventionalcancer therapy with immunotherapy. Nat Rev Cancer. 2017; 17: 286-301. DOI: https://doi.org/10.1038/nrc.2017.17
30. Brown JS, Sundar R, Lopez J. Combining DNA damaging therapeutics with immunotherapy: More haste, less speed. Br J Cancer. 2018; 118: 312-324. DOI: https://doi.org/10.1038/bjc.2017.376
31. Zhang P, Su DM, Liang M, Fu J. Chemopreventive agents induce programmed death-1-ligand 1 (PD-L1) surface expression in breast cancer cells and promote PD-L1-mediated T cell apoptosis. Mol Immunol. 2008; 45: 1470-1476. DOI: https://doi.org/10.1016/j.molimm.2007.08.013
32. Pelekanou V, Carvajal-Hausdorf DE, Altan M, et al. Effect of neoadjuvant chemotherapy on tumor-infiltrating lymphocytes and PD-L1 expression in breast cancer and its clinical significance. Breast Cancer Res. 2017; 19: 91. DOI: https://doi.org/10.1186/s13058-017-0884-8