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Correlation of HLA-A and HLA-B/C Expression With ESR1 Expression in Patients With Metastatic Breast Cancer as a Potential Prognosticator of Favorable Distant Disease-free Survival

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Abstract. *Background/Aim: The loss of breast cancer cell differentiation during metastatic progression leads to a down-regulation of class 1 human leukocyte antigen (HLA) expression, which in turn hinders cytotoxic T lymphocytes from effectively preventing tumor cell proliferation. Consequently, one would expect that decreased HLA expression would correlate with decreased 5-year survival. However, estrogen receptor alpha (ESR1) is known to be positively associated with overall survival. The study aimed to determine the expression levels of HLA-A, HLA-B/C, and ESR1 and to assess their influence on distant disease-free survival (DDFS). Materials and Methods: This retrospective subgroup analysis of the initial prospective, single-center, double-blind cohort study included a total of 34 patients who underwent a new treatment line for metastatic breast cancer (MBC). The MBC cells were examined using RT-qPCR. Results: The acquired data and the subsequent survival and ROC analyses indicated a positive association of reduced expression of HLA-A and HLA-B/C with DDFS. A statistically significant association of ESR1 with DDFS could not be shown. Conclusion: A potential positive association between reduced expression of HLA-A and HLA-B/C and DDFS is observed. This contrasts with the generally observed association between HLA expression loss and poor prognosis, as reported in previous protein-based studies. In metastatic settings, reduced expression of particular HLA subsets, measured at the mRNA level, might have a protective effect against disease progression.*

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Breast cancer remains one of the most common cancers in women worldwide, with metastatic breast cancer (MBC) being one of the leading causes of cancer-related deaths among solid malignancies (1-3). New forms of therapy such as antibody-drug

Table I. *Clinicopathological patient and tumor characteristics (n=34).*

Characteristic	N	Value
Age at initial BC diagnosis, median (range), years	34	50 (34-73)
Age at study enrolment, median (range), years	34	55 (42-80)
Metastasis site		
Bone, frequency (%)		7 (20.6%)
Liver, frequency (%)		5 (14.7%)
Lung, frequency (%)	34	2 (5.9%)
Brain, frequency (%)		2 (5.9%)
Other, frequency (%)		18 (52.9%)
Tumor grade		
Grade 1, frequency (%)		16 (47.1%)
Grade 2, frequency (%)	34	9 (26.5%)
Grade 3, frequency (%)		0 (0%)
Unknown, frequency (%)		9 (26.5%)
Intrinsic tumor subtype, mRNA-based		
Luminal A, frequency (%)		12 (35.3%)
Luminal B, frequency (%)	34	13 (38.2%)
Triple negative, frequency (%)		7 (20.6%)
Unknown, frequency (%)		2 (5.9%)
DDFS, median (range), months	34	61.5 (0-190)
ESR1 40-ΔΔCT, median (range)	34	40.3 (34.8-43.5)
OS after breast cancer diagnosis, median (range), months	34	114 (2-260)
OS after study enrolment, median (range), months	34	33.5 (1-156)
Death until end of study or loss to follow-up, frequency (%)	34	29 (85.3%)

BC: Breast cancer; DDFS: distant disease-free survival; OS: overall survival.

conjugates have been introduced and breast cancer subtyping has improved diagnosis and prognosis assessment (4-7). However, despite rapid scientific progress in this field, women with MBC still do not have a sufficiently favorable prognosis (4).

Class I HLA are molecules that occur on almost all human cells. One of their most important functions is tumor antigen presentation to cytotoxic T lymphocytes (CTL) (8). If these tumor antigens are presented to CTL, they are usually able to recognize and destroy the tumor cells (9-11). As part of the de-differentiation of tumor cells, a reduction or even complete loss of HLA can occur (12-14). It is logical that a reduction in HLA and the associated reduced activation of CTL leads to an increased spread of the tumor and thus, to a worse course of the disease for the patient, with reduced DDFS (9, 15). In contrast, it is well known that increased expression of ESR1 has a positive effect on the overall outcome (16-20). The aim of this study was to correlate the expression levels of HLA-A, HLA-B/C, and ESR1 with each other to derive a model that provides a more reliable prediction of the 5-year survival of patients with MBC.

Materials and Methods

In this retrospective subgroup analysis of the initial prospective, double-blind cohort study, 34 patients treated for MBC at the German National Center for Tumor Diseases (NCT), University of Heidelberg, Germany, between March 2010 and May 2015, were analyzed. We examined the expression levels of HLA-A, HLA-B/C, and ESR1 and their influence on the DDFS of the patients. The measurements were obtained from distant metastatic tissue, RT-qPCR technology was used to determine the expression levels of HLA-A, HLA-B, HLA-C, and ESR1 in breast cancer cells, as along with their intrinsic subtypes, utilizing *CALM2* as the housekeeping gene, according to well-established protocols (16). Receiver operating characteristic (ROC) curves were constructed to assess the sensitivity and specificity of the analyzed parameters in relation to their predictive value for DDFS. To this end, Youden J points were calculated for the ROC curves with consecutive determination of sensitivity and specificity at the respective J points. All statistics were calculated using R (R Foundation for Statistical Computing, Vienna, Austria, version 3.1.2). Additionally, Kaplan–Meier curves were generated to evaluate the impact of individual parameters on DDFS. The relative gene expression is presented as 40-ΔΔCT with higher values corresponding to higher mRNA counts in the tissue biopsy sample. Details on progression restaging regimen, double-blinding, utilization of electronic health records and patient demographics have been extensively reported in recent publications of our collaborative group (21-24).

Results

The average age of the 34 patients included in the study was 55 years, and 50 years at the time of initial diagnosis. The median DDFS was 61.5 months. The median OS after inclusion in the study was 33.5 months. The most common intrinsic tumor subtype was luminal B, and the most common site of distant metastasis was the bone. A total of 29 of the 34 patients had

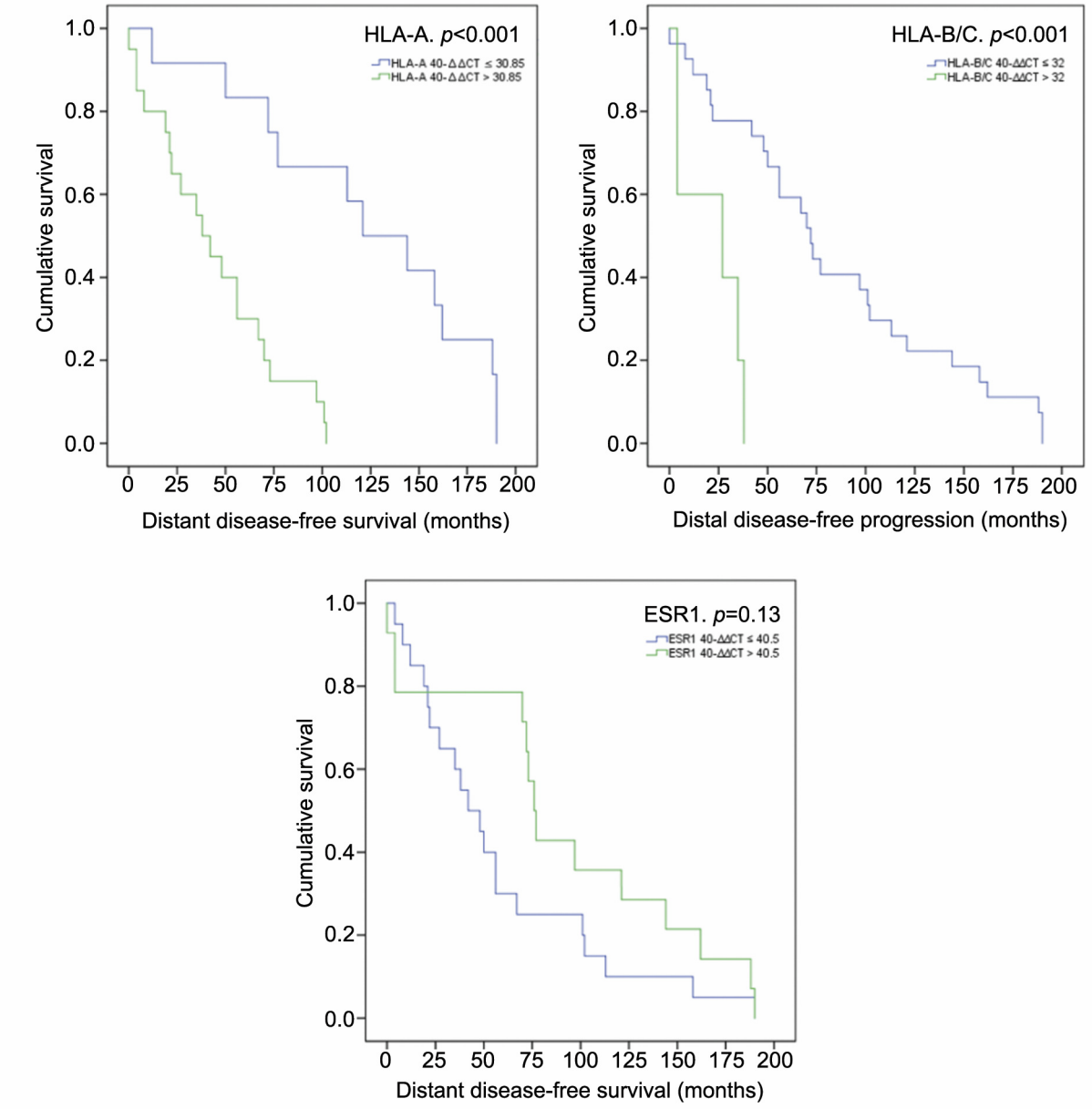

Figure 1. *Kaplan–Meier analysis of distant disease-free survival based on mRNA expression of A) HLA-A (n=32), B) HLA-B/C (n=32), and C) ESR1 (n=34).*

died or were lost to follow-up at the end of the study. Table I provides a detailed list of the patients and tumor characteristics.

Figure 1 shows Kaplan–Meier curves for the DDFS of ESR1, HLA-A, and HLA-B/C. The data indicate that HLA-A and HLA-B/C are significantly associated with DDFS, whereas no such association is observed for ESR1. Figure 2 shows the ROC curves for the examined parameters. Using the ROC curves, the sensitivity and specificity of the individual parameters in relation to the predictive value for DDFS are visualized. For better understanding, the effective values of sensitivity and specificity for ESR1, HLA-A, and HLA-B/C are shown separately in Table II.

Discussion

This cohort shows that reduced expression of HLA-A and HLA-B/C has a positive influence on DDFS in the metastatic situation. This means that in the case of the metastatic situation, the signs of the association of HLA-A and HLA-B/C with the DDFS appear to be reversed from the previously reported increased expression having a positive impact on DDFS and overall survival (OS) in patients with breast cancer, when measured by routine protein-based assays (9, 15). However, this study shows

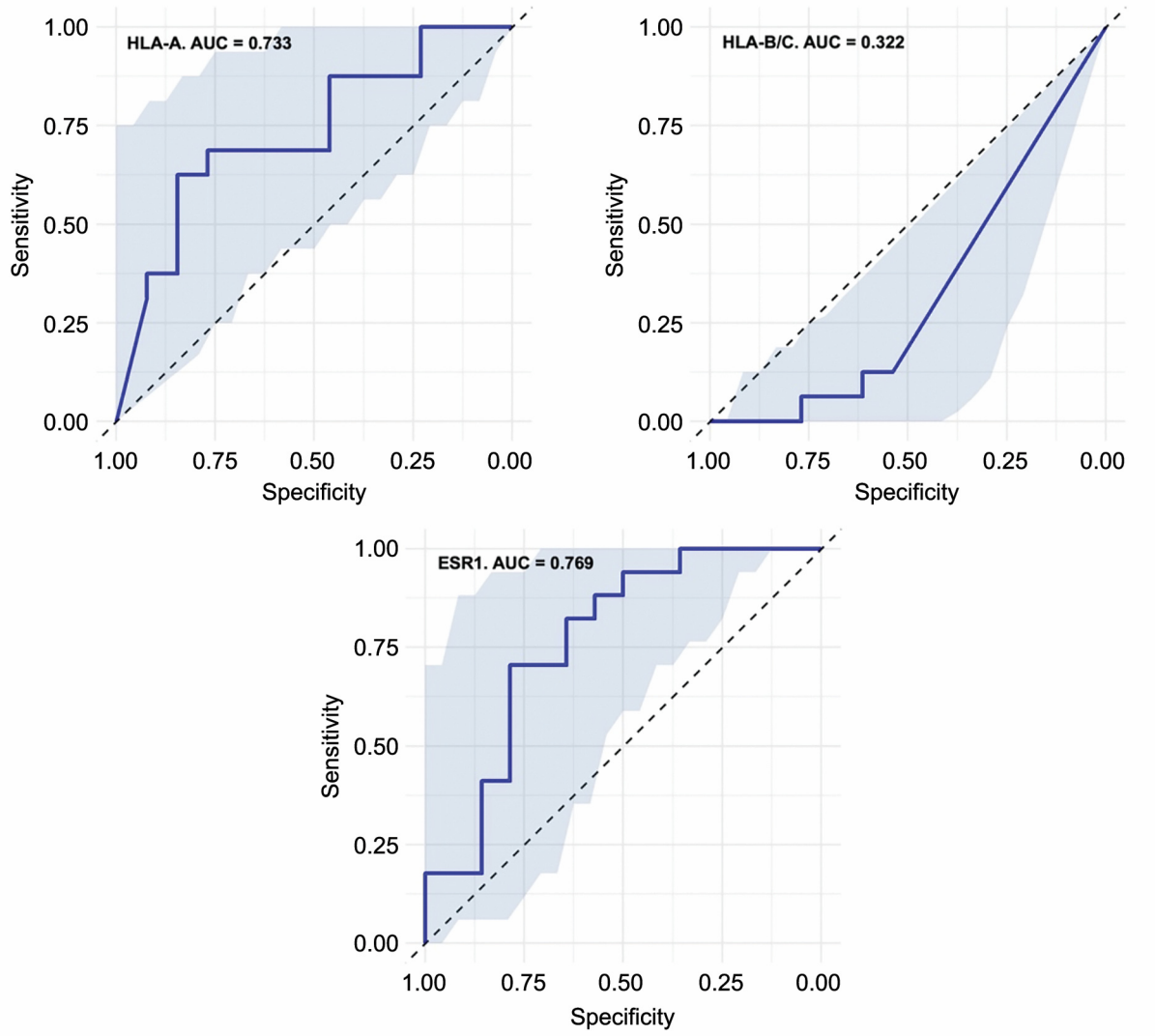

Figure 2. *Distant disease-free survival (DDFS)-related ROC curves representing the sensitivity and specificity of A) HLA-A, B) HLA-B/C, and C) ESR1 expression for detection of patients that will not experience distant disease progression within five years (n=32). The light blue areas signify the 95% confidence interval.*

Table II. *The effective values of sensitivity and specificity for ESR1, HLA-A, and HLA-B/C.*

Cut-off value determination strategy	Cut-off value (40-ΔΔCT)	Sensitivity	Specificity
ESR1 (5-year DDFS)			
Youden J point	40.5	70.6%	78.8%
Optimized for maximum sensitivity	38.4	94.1%	50%
Optimized for maximum specificity	42.6	17.7%	92.9%
HLA-A (5-year DDFS)			
Youden J point	30.85	62.5%	84.6%
Optimized for maximum sensitivity	33.6	93.8%	23.1%
Optimized for maximum specificity	30.3	37.5%	92.3%
HLA-B/C (5-year DDFS)			
Youden J point	-∝	ø	ø
Optimized for maximum sensitivity	30.8	12.5%	61.5%
Optimized for maximum specificity	32	6.3%	76.9%

DDFS: Distant disease-free survival.

that this behaves differently in the metastatic situation, at least with regard to the DDFS when looking particularly into the HLA-A and B/C subsets at the mRNA level. Here, reduced expression of Class I HLA appears to have a positive influence on DDFS. Due to the limiting factor of fairly small sample size and the methodic differences in terms of looking into particular HLA subsets by RT-qPCR *versus* previous bulk-HLA antibody stainings, we underline the proof-of-concept and hypothesis generating character of our study One hypothesis could be that the absence of CTLs gives other immune cells increased access to the tumor cells and thereby curbs progression, although whether this is really the case will have to be shown by other, more extensive studies.

The expression of ESR1 is known to be positively associated with DDFS and OS. In our cohort, the influence of ESR1 on DDFS did not turn out significant. This is in contrast to a wide range of previous studies on the topic (16-18). The question therefore arises as to whether the signs are reversed in the metastatic situation regarding ESR1 and its association with DDFS or whether a statistically significant association with DDFS cannot be shown simply because of the very small patient cohort. Because the influence of ESR1 is not significantly associated with DDFS in our cohort, this does not allow a combination of the 3 examined parameters (HLA-A, HLA-B/C, ESR1) to improve the prediction of the DDFS.

With only 34 patients fulfilling very demanding criteria in terms of tissue availability from distant metastatic sites, intrinsic subtype and mRNA expression analysis of surface molecules and loss to follow-up, our study is subject to statistical difficulties, which are ultimately reflected in a non-significant association of ESR1 with DDFS and thus do not allow a conclusive assessment of the relationships. We therefore plan a larger multi-center study in order to include significantly more patients and to finally clarify the aforementioned hypotheses. However, it is certain that HLA can be an important prognostic factor for estimating the clinical course of the disease. Whether the prognostic value of HLA can best be supported by ESR1 or whether other markers could prove to be more sufficient remains unclear and must be part of further investigations.

Conclusion

As anticipated, Class I HLA appears to be a possible valuable predictor of DDFS in patients with MBC. The trends observed in our HLA-subset analysis at the mRNA level are reversed; contrary to expectations, lower expression of HLA-A and HLA-B/C is positively associated with DDFS. Whether ESR1 can enhance the predictive value of HLA cannot be conclusively clarified and requires further investigation through larger, more comprehensive studies.

Conflicts of Interest

R. W. is an employee of Stratifyer Molecular Pathology GmbH. All other Authors have nothing to disclose in relation to this study.

Authors' Contributions

Conception and design of the study: J.H., S.S., M.W. ; Data collection: R.W., A.S. T.M.D.; Data analysis & interpretation: U.K., S.S., A.S. L.G.; Statistical analysis: U.K., L.G.; Manuscript preparation phase 1 – drafting the article: L.G. S.S., U.K., M.K.; Manuscript preparation phase 2- revising it critically for important intellectual content: M.W., M.S., R.W., J.H.; Final approval of the version to be submitted: all Authors.

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