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An Integrated Circulating Biomarker Index for Overall Survival in Metastatic Breast Cancer Treated with Chemotherapy: A Retrospective Cohort Study.

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Abstract

Background

Circulating biomarkers associated with tumor–immune interactions and clinical outcomes in breast cancer are generally evaluated individually. We investigated their prognostic significance in patients with metastatic breast cancer (MBC) receiving chemotherapy and developed an integrated biomarker index for overall survival (OS).

Methods

We retrospectively analyzed 43 patients with MBC who were receiving chemotherapy. Baseline circulating levels of tumor necrosis factor-α (TNF-α), soluble interleukin-2 receptor (sIL-2R), interleukin-6 (IL-6), IL-8, transforming growth factor-β (TGF-β), and thymidine kinase 1 (TK1) were measured. Biomarkers associated with OS were combined to construct the OS Integrated Biomarker Index (OS-IBI). Associations of the OS-IBI with survival and peripheral immune and inflammatory parameters were evaluated.

Results

In univariable Cox proportional hazards analysis, TNF-α ($p = 0.008$), sIL-2R ($p < 0.001$), IL-6 ($p = 0.001$), and TK1 ($p = 0.003$) were significantly associated with shorter OS and were subsequently incorporated into the OS-IBI. The OS-IBI showed numerically greater discrimination for OS than any individual biomarker (AUC 0.801, 95% CI: 0.658–0.944) and remained independently associated with shorter OS in multivariable analysis (HR 1.82, 95% CI: 1.31–2.53; $p < 0.001$). Bootstrap internal validation yielded an optimism-corrected C-index of 0.817. Higher OS-IBI values were associated with lower proportions of $CD4^+$ lymphocytes and higher proportions of myeloid-derived suppressor cells, as well as unfavorable systemic inflammatory parameters.

Conclusions

The OS-IBI, integrating circulating biomarkers related to inflammation, immune activation, and tumor proliferation, was independently associated with OS and with peripheral features of systemic immune dysregulation. These findings warrant validation in larger, independent cohorts.

Introduction

Despite recent advances in systemic therapies, metastatic breast cancer (MBC) remains an incurable disease, and chemotherapy continues to play an important role in its management. However, clinical outcomes vary considerably among patients, highlighting the need for reliable biomarkers to improve

prognostic stratification and inform treatment strategies. The tumor microenvironment has emerged as a critical determinant of tumor progression and therapeutic resistance [1, 2]. Cytokines mediate interactions between tumor cells and the immune system and contribute to the establishment of an immunosuppressive tumor microenvironment [3, 4]. Dysregulated cytokine signaling may impair anti-tumor immunity and promote the expansion of immunosuppressive cell populations, thereby contributing to unfavorable clinical outcomes in various malignancies, including breast cancer [5]. For example, elevated baseline interleukin (IL)-6 levels have been associated with poor prognosis in patients with MBC receiving eribulin [6]. Other circulating cytokines and soluble mediators, including soluble IL-2 receptor (sIL-2R), tumor necrosis factor-α (TNF-α), IL-8, and transforming growth factor-β (TGF-β), have also been associated with breast cancer progression or clinical outcomes [7–10]. In addition, thymidine kinase 1 (TK1), a marker of cellular proliferation, has been associated with survival outcomes in patients with breast cancer [11].

Previous studies have used multiplex assays to simultaneously characterize multiple circulating cytokines and examine their associations with clinicopathological features and clinical outcomes in patients with breast cancer [12, 13]. Notably, circulating cytokine profiles and cytokine clusters have been associated with aggressive tumor characteristics and prognosis, suggesting that a combined assessment may capture biological information not represented by individual biomarkers alone [12]. In parallel, large-scale genomic datasets have enabled the development of prognostic signatures based on immune- and cytokine-related gene expression profiles in breast cancer [14–16]. However, the prognostic utility of integrating circulating protein biomarkers into a composite index, and the relationship of such an index with systemic immune alterations, remain incompletely understood. A blood-based approach may complement tissue-based molecular profiling while providing a minimally invasive assessment of systemic biological alterations.

In the present study, we systematically evaluated circulating biomarkers in patients with MBC receiving chemotherapy, focusing on cytokine- and inflammation-related factors, together with TK1 as a marker of cellular proliferation. We investigated the prognostic significance of individual biomarkers and subsequently developed an overall survival (OS) Integrated Biomarker Index (OS-IBI) based on biomarkers associated with OS. Furthermore, we examined the associations between the OS-IBI and peripheral immune and inflammatory parameters to explore its biological relevance and potential relationship with systemic immune dysregulation.

Patients and Methods

Study design

This retrospective analysis included 43 patients with MBC who underwent chemotherapy at Hyogo Medical University Hospital between June 2021 and November 2023. Patients were identified from the medical records of all patients who initiated chemotherapy for MBC at our institution during the study period. Eligible patients had received at least one cycle of chemotherapy; to minimize selection bias, all

consecutive patients meeting this criterion were included. No formal sample size calculation was performed because of the retrospective exploratory nature of the study. Follow-up data were obtained from medical records, and survival status was confirmed as of April 2025. Breast cancer was histologically confirmed in all patients, and the presence of metastasis was verified by diagnostic imaging, including computed tomography, whole-body bone scintigraphy, or 2-[^{18}F]fluoro-2-deoxy-D-glucose positron emission tomography.

Tumors were categorized according to the combined estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2) status. ER negativity was defined as < 1% positive tumor cell nuclei with appropriate internal (normal epithelial tissue) and external control reactivity on immunohistochemical staining. HER2-negative status was assigned to tumors with immunohistochemistry scores of 0 or 1+, or 2+, without evidence of HER2 gene amplification as determined by in situ hybridization. The study protocol was reviewed and approved by the Institutional Review Board of Hyogo College of Medicine (Approval Nos. 1969 and 106). All study procedures adhered to the Declaration of Helsinki, and written informed consent was obtained from each participant.

Measurements of peripheral blood biomarkers

Baseline peripheral blood samples were collected on the day before the initiation of the index chemotherapy regimen. Serum levels of TNF-α, sIL-2R, IL-6, and TK1, and plasma levels of IL-8 and TGF-β, were quantified at an external clinical laboratory (SRL Inc., Tokyo, Japan) by personnel blinded to clinical outcomes. Serum TNF-α and TK1 levels were measured using enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, MN, USA, and DiaSorin, Saluggia, Italy, respectively). Serum sIL-2R and IL-6 levels were measured using chemiluminescent enzyme immunoassays (Fujirebio Inc., Tokyo, Japan). Plasma IL-8 and TGF-β levels were measured using enzyme-linked immunosorbent assays (Invitrogen, Carlsbad, CA, USA, and R&D Systems, respectively).

Neutrophil, lymphocyte, monocyte, and platelet counts were measured automatically using a Sysmex XN-9000 or XN-1000 hematology analyzer (Sysmex Corp., Kobe, Japan). The absolute lymphocyte count (ALC) was calculated by multiplying the total white blood cell count by the lymphocyte percentage. The neutrophil-to-lymphocyte ratio (NLR) was defined as the neutrophil count divided by the lymphocyte count. The lymphocyte-to-monocyte ratio (LMR) was defined as the lymphocyte count divided by the monocyte count.The prognostic nutritional index (PNI) was calculated using the conventional formula: PNI = 10 × serum albumin (g/dL) + 0.005 × peripheral blood absolute lymphocyte count (/mm ^{3}) [17]. Lactate dehydrogenase (LDH) was measured using a Cobas 8000 c702 module (Roche Diagnostics, Tokyo, Japan). C-reactive protein (CRP) was measured using a LABOSPECT 008 analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan).

Flow cytometry analysis

Flow cytometric analyses were performed for research purposes on freshly obtained peripheral blood samples from patients who provided written informed consent prior to chemotherapy. These analyses were conducted at baseline, concurrently with the cytokine measurements. Peripheral blood samples were collected into tubes, diluted with Dulbecco's phosphate-buffered saline without calcium and magnesium (D-PBS (−); Nacalai Tesque, Kyoto, Japan), and carefully layered onto Ficoll–Paque PLUS (Cytiva, Marlborough, MA, USA) using SepMate-50 tubes (STEMCELL Technologies, Vancouver, Canada). The samples were then centrifuged at 1200 × *g* for 20 min at 20°C. The mononuclear cell-enriched layer was subsequently harvested and transferred to a new tube, followed by centrifugation at 300 × *g* for 10 min at 20°C for washing.

Residual red blood cells were lysed by treatment with 1× RBC Lysis Buffer (Thermo Fisher Scientific, Waltham, MA, USA); the cells were washed twice with D-PBS (−). Cell numbers were determined using a Countess 2 FL Automated Cell Counter (Thermo Fisher Scientific), and the cells were then subjected to flow cytometric analysis.

Prior to antibody staining, the cells were incubated with human AB serum (GemCell, Seven Hills, Australia) for 30 min at 4°C in the dark to block nonspecific binding. The cells were subsequently stained with fluorochrome-conjugated monoclonal antibodies for 30 min at 4°C in the dark. After staining, the samples were washed with Cell Staining Buffer (BioLegend, San Diego, CA, USA) and fixed using True-Nuclear 1× Fix Concentrate (BioLegend) for 15 min at room temperature in the dark for surface marker analysis or for 45 min at room temperature for intracellular staining. Cells were further incubated with conjugated monoclonal antibodies diluted in True-Nuclear 1× Perm Buffer (BioLegend) for 30 min for intracellular marker detection. Data acquisition was performed using a BD LSRFortessa X-20 flow cytometer (BD Biosciences, San Jose, CA, USA), and the results were analyzed using BD FACSDiva software. Flow cytometric analyses were performed by investigators blinded to survival outcomes.

Results

Clinical Outcomes According to Individual Biomarkers for OS

An overview of the patient characteristics is presented in Supplementary Table S1. Of the 43 enrolled patients, one patient with unknown HER2 status was excluded from the Cox proportional hazards analyses presented in Table 2 and Supplementary Table S3; therefore, 42 patients were included in these analyses. The median follow-up duration was 24.0 months (95% CI: 21.0–30.3 months), as estimated using the reverse Kaplan–Meier method. During follow-up, 16 deaths were observed. Among the individual biomarkers, IL-6 demonstrated the highest discriminative performance for OS event status (AUC 0.751, 95% CI: 0.593–0.910), followed by TK1 (AUC 0.734, 95% CI: 0.580–0.888), TNF-α (AUC 0.715, 95% CI: 0.560–0.871), and sIL-2R (AUC 0.674, 95% CI: 0.501–0.846) (Table 1, Fig. 1a). In univariable Cox proportional hazards analysis, TNF-α (HR 3.88, 95% CI: 1.42–10.61, $p = 0.008$), sIL-2R (HR 7.16, 95% CI: 2.24–22.88, $p < 0.001$), IL-6 (HR 2.24, 95% CI: 1.37–3.65, $p = 0.001$), and TK1 (HR 1.81,

95% CI: 1.23–2.67, $p = 0.003$) were significantly associated with OS, whereas IL-8 and TGF-β were not (Table 1).

Table 1
Univariable analysis and discriminative performance of individual biomarkers for overall survival

Variable	HR (95% CI)	*p* value	AUC (95% CI)
TNF-α	3.88 (1.42–10.61)	0.008	0.715 (0.560–0.871)
sIL-2R	7.16 (2.24–22.88)	< 0.001	0.674 (0.501–0.846)
IL-6	2.24 (1.37–3.65)	0.001	0.751 (0.593–0.910)
IL-8	1.63 (0.98–2.71)	0.059	0.545 (0.366–0.725)
TGF-β	1.72 (0.79–3.72)	0.171	0.513 (0.332–0.694)
TK1	1.81 (1.23–2.67)	0.003	0.734 (0.580–0.888)

Table 2
Univariable and multivariable Cox proportional hazards analyses of the Overall Survival Integrated Biomarker Index (OS-IBI) and clinical covariates for overall survival.

		Univariable analysis		Multivariable analysis	
	n	HR (95% CI)	*p* value	HR (95% CI)	*p* value
OS-IBI	42	1.63 (1.25–2.11)	< 0.001	1.82 (1.31–2.53)	< 0.001
Treatment lines					
First line	24	1		1	
≥ 2 lines	18	3.44 (1.05–11.27)	0.041	1.13 (0.33–3.89)	0.852
Estrogen receptor					
ER-negative	19	1		1	
ER-positive	23	0.88 (0.29–2.66)	0.820	0.86 (0.19–3.90)	0.847
HER2					
HER2-negative	30	1		1	
HER2-positive	12	0.45 (0.12–1.63)	0.230	0.16 (0.03–0.93)	0.042

Development and Validation of the OS Integrated Biomarker Index

Based on biomarkers significantly associated with OS, the OS-IBI was constructed using TNF-α, sIL-2R, IL-6, and TK1. The OS-IBI demonstrated numerically better discriminative performance for OS than any

individual biomarker (AUC 0.801, 95% CI: 0.658–0.944) (Fig. 1a), although the confidence intervals overlapped. In internal validation using 1,000 bootstrap resamples, IL-6, sIL-2R, TK1, and TNF-α were selected in 94.9%, 93.9%, 82.7%, and 72.7% of resamples, respectively, compared with 43.8% for IL-8 and 24.4% for TGF-β. Although the exact four-biomarker combination was reproduced in only 21.3% of resamples, each of the four constituent biomarkers was selected frequently. Despite this variability, discriminative performance was largely maintained after correction for optimism. The apparent C-index, defined as Harrell's C-index of the final OS-IBI evaluated in the original cohort, was 0.840. After bootstrap correction, the optimism-corrected C-index was 0.817 (mean optimism, 0.023) (Fig. 1b). When analyzed as a continuous variable, the OS-IBI was significantly associated with shorter OS in both univariable (HR 1.63, 95% CI: 1.25–2.11; $p < 0.001$) and multivariable Cox proportional hazards analyses (HR 1.82, 95% CI: 1.31–2.53; $p < 0.001$) (Table 2). Among the clinical covariates, HER2-positive status was independently associated with improved OS (HR 0.16, 95% CI: 0.03–0.93; $p = 0.042$), whereas treatment line and ER status were not significantly associated with survival. Kaplan–Meier analysis demonstrated significantly shorter OS in the high-OS-IBI group than in the low-OS-IBI group when dichotomized at the median (log-rank $p < 0.001$) (Fig. 1c).

Subgroup Analysis of the OS-IBI for OS

The prognostic association between the OS-IBI and OS was further evaluated across clinically relevant subgroups. In the overall population (n = 43), a higher OS-IBI was associated with an increased risk of death (HR 1.62, 95% CI: 1.26–2.09; $p < 0.001$). Subgroup analyses according to menopausal status, recurrent versus *de novo* advanced disease, treatment line, ER status, HER2 status, and drug class showed no statistically significant evidence of heterogeneity in the association between the OS-IBI and OS (all p for interaction > 0.05). These findings suggest that the association between higher OS-IBI values and shorter OS was generally maintained across the evaluated clinical subgroups (Fig. 2).

Association of the OS-IBI With Immune and Inflammatory Parameters

The biological relevance of the OS-IBI was further explored by examining its associations with immune and inflammatory parameters. The OS-IBI was negatively correlated with the proportion of $CD4^+$ lymphocytes (% of PBMCs), ALC, LMR, and PNI, and positively correlated with the proportion of MDSCs (% of PBMCs), LDH, and CRP, whereas no significant correlations were observed with $CD8^+$ lymphocytes, Tregs or NLR (Fig. 3).

Exploratory Analysis of Individual Biomarkers for PFS

A total of 29 PFS events were observed. Among the evaluated biomarkers, TGF-β was the only biomarker significantly associated with PFS in univariable Cox proportional hazards analysis of natural log-transformed values (HR 1.78, 95% CI: 1.01–3.12; $p = 0.046$) (Supplementary Table S2). However, its discriminative performance for PFS was modest (AUC 0.597, 95% CI: 0.410–0.785) (Supplementary

Fig. 1a). For further analysis, the natural log-transformed TGF-β values were standardized as z-scores. Among the 42 patients with complete covariate data, higher standardized TGF-β levels were significantly associated with shorter PFS in both univariable (HR 1.50, 95% CI: 1.01–2.22; $p = 0.044$) and multivariable Cox proportional hazards analyses (HR 1.55, 95% CI: 1.01–2.38; $p = 0.044$) (Supplementary Table S3). Kaplan–Meier analysis using the median TGF-β level as the cutoff demonstrated significantly shorter PFS in patients with high TGF-β levels than in those with low TGF-β levels (log-rank $p = 0.028$) (Supplementary Fig. 1b). No significant correlations were observed between TGF-β and any of the evaluated immune or inflammatory parameters (Supplementary Fig. 2).

Discussion

Considering the biological heterogeneity of cytokine signaling and the complexity of immune regulation in patients receiving chemotherapy, reliance on a single circulating biomarker may be insufficient to capture clinically relevant systemic biological alterations in patients with MBC. An integrative assessment of multiple circulating biomarkers may therefore provide a more comprehensive representation of host–tumor interactions than individual biomarkers alone. Previous studies have demonstrated the prognostic significance of circulating cytokines and other blood-based biomarkers in MBC; however, the clinical relevance of integrating multiple biomarkers remains unclear. In the present study, elevated baseline TNF-α, sIL-2R, IL-6, and TK1 were associated with shorter OS. Based on these findings, we developed the OS-IBI, which demonstrated numerically greater discrimination for OS than any individual biomarker and remained independently associated with OS after adjusting for clinicopathological factors. Furthermore, higher OS-IBI values were associated with lower proportions of $CD4^+$ lymphocytes and higher proportions of MDSCs, as well as with several unfavorable systemic immune and inflammatory parameters. These findings suggest that the OS-IBI may integrate biological information related to systemic inflammation, immune dysregulation, and tumor proliferation and may reflect an immunosuppressive state associated with poor clinical outcomes.

In this cohort, higher baseline concentrations of TNF-α, sIL-2R, IL-6, and TK1 were significantly associated with shorter OS, whereas IL-8 and TGF-β levels were not significantly associated with OS. These findings are broadly consistent with those of previous reports. The associations of elevated TNF-α and TK1 with poorer OS are consistent with earlier studies [11, 20], as are the associations of sIL-2R and IL-6 with poorer OS [6, 20, 21]. Although IL-8 and TGF-β were not statistically associated with OS in the present study, the direction of their associations was consistent with that reported previously [8, 10, 22], suggesting a possible but weaker effect. Collectively, these findings support the prognostic relevance of circulating immune and proliferative biomarkers in patients with MBC.

We next integrated the four biomarkers associated with OS into the OS-IBI. The OS-IBI showed numerically greater discrimination for OS than any individual biomarker, and higher OS-IBI values were independently associated with shorter OS. Moreover, the association between higher OS-IBI values and poorer OS was generally consistent across clinically relevant subgroups, with no significant interactions. Previous studies have integrated cytokine-related molecular information to develop prognostic

signatures in breast cancer using transcriptomic datasets [14–16]. However, relatively few studies have examined the combined prognostic value of multiple circulating protein biomarkers, particularly in patients with MBC receiving chemotherapy [12, 23]. Because circulating biomarkers can be assessed using minimally invasive blood sampling, integrated blood-based biomarker approaches may be suitable for clinical applications [23]. In the present study, the OS-IBI showed favorable prognostic performance, suggesting that integrating multiple biomarkers may provide more prognostic information than evaluating individual biomarkers alone.

We further examined the biological relevance of the OS-IBI by assessing its associations with peripheral immune and inflammatory parameters. Higher OS-IBI values were associated with lower proportions of $CD4^+$ lymphocytes and higher proportions of MDSCs, as well as lower ALC, LMR, and PNI, and higher LDH and CRP levels. MDSCs are immunosuppressive cell populations that contribute to impaired anti-tumor immune responses. In patients with advanced breast cancer, circulating MDSCs and other immune-cell populations have been associated with clinical outcomes and treatment response [24]. Moreover, higher frequencies of MDSCs have been associated with impaired tumor-reactive T-cell responses and poorer survival in patients with breast cancer [25]. Peripheral blood inflammatory parameters, including NLR and ALC, have also been associated with treatment efficacy and prognosis in patients with breast cancer [26–30]. Notably, a recent study of patients with advanced breast cancer receiving chemotherapy demonstrated that lower ALC, higher NLR, and lower LMR were associated with higher circulating MDSC levels [31]. These observations are consistent with our finding that higher OS-IBI values were associated with both increased MDSCs and unfavorable peripheral blood parameters. Collectively, these findings suggest that the OS-IBI may capture systemic biological alterations associated with an immunosuppressive state and adverse clinical outcomes, although the underlying mechanisms remain to be elucidated.

As an exploratory analysis, we examined the association between individual biomarkers and PFS. Among the evaluated biomarkers, only elevated TGF-β was significantly associated with shorter PFS. This finding is consistent with previous studies that showed an association between elevated circulating TGF-β levels and adverse clinical outcomes in patients with breast cancer [8, 32]. In addition, increased activity of the TGF-β signaling pathway has been associated with unfavorable PFS in patients with breast cancer [33]. TGF-β has pleiotropic roles in cancer progression, including the promotion of immunosuppression, epithelial–mesenchymal transition, and metastasis, which may partly explain its association with shorter PFS. However, the prognostic significance of TGF-β has not been consistently demonstrated across studies. In the TRANSERI-GONO study, baseline plasma TGF-β was not significantly associated with PFS in patients with metastatic breast cancer treated with eribulin [34]. Similarly, Li et al. reported no significant association between tumor TGF-β1 expression and PFS in a cohort of 417 patients with invasive breast cancer [35]. These discrepant findings may reflect differences in patient populations, disease subtypes, treatment settings, and methods used to assess TGF-β, and the prognostic significance of TGF-β in breast cancer therefore remains inconclusive.

This study had several limitations. First, its retrospective, single-center design and relatively small sample size (43 patients; 16 deaths) may limit the statistical power and generalizability and increase the risk of model overfitting, particularly because the index was developed and evaluated within the same cohort. Although bootstrap resampling with variable selection repeated within each resample was used for internal validation, biomarker selection was not perfectly stable across resamples, and further validation in independent cohorts would strengthen the generalizability of our findings. Second, heterogeneity in chemotherapy regimens and treatment lines may have influenced biomarker levels and clinical outcomes. Third, biomarkers were assessed only at baseline, and PFS was evaluated retrospectively without a prospectively standardized imaging schedule. Finally, the observational design precludes causal inference, and residual confounding and other sources of bias inherent to retrospective analyses cannot be excluded. Despite these limitations, our findings support the potential prognostic utility of the OS-IBI and provide insights into its biological basis through its associations with systemic inflammation, immune status, and tumor proliferative activity. These findings provide a rationale for further investigation of the OS-IBI in larger prospective cohorts.

In conclusion, a systematic evaluation of circulating biomarkers in patients with MBC receiving chemotherapy identified biomarkers associated with OS that were integrated into the OS-IBI. The OS-IBI was independently associated with OS and was also associated with peripheral immune and inflammatory parameters, including circulating MDSCs and $CD4^+$ lymphocytes. These findings suggest that the OS-IBI may capture not only prognostic information but also systemic biological features related to inflammation and immune dysregulation in MBC.

Declarations

Author contributions

Junko Tsuchida: Conceptualization, Funding acquisition, Investigation, Writing – original draft. **Masayuki Nagahashi**: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft. **Sayaka Urano**: Investigation, Writing – original draft. **Mamiko Kuroiwa**: Investigation, Writing – review & editing. **Miki Komatsu**: Investigation, Methodology, Writing – original draft. **Shoko Yoshida**: Investigation, Writing – review & editing. **Aoi Oshiro**: Investigation, Writing – review & editing. **Gen Sugimoto**: Investigation, Writing – review & editing. **Ayumu Mitsuyoshi**: Investigation, Writing – review & editing. **Yusa Togashi**: Investigation, Writing – review & editing. **Haruka Kanaoka**: Investigation, Writing – review & editing. **Akira Hattori**: Investigation, Writing – review & editing. **Tomoko Higuchi**: Investigation, Writing – review & editing. **Arisa Nishimukai**: Investigation, Writing – review & editing. **Toshifumi Wakai**: Supervision, Writing – review & editing. **Masafumi Shimoda**: Supervision, Writing – review & editing. **Yasuo Miyoshi**: Project administration, Supervision, Writing – review & editing.

Compliance with Ethical Standards

Disclosure of conflicts of interest

M. Nagahashi received honoraria from Chugai, AstraZeneca, Eli Lilly, Pfizer, Novartis, MSD, Taiho, Daiichi Sankyo, Eisai, Kyowa-Kirin, and Denka. **Y. Miyoshi** received research funding and honoraria from Eisai, Chugai, AstraZeneca, Eli Lilly, Pfizer, MSD, Kyowa-Kirin, Daiichi Sankyo, and Taiho. **M. Shimoda** received honoraria from Chugai, Pfizer, MSD, Eisai, AstraZeneca, Eli Lilly, Daiichi Sankyo, and Kyowa-Kirin. **J. Tsuchida, S. Urano, M. Kuroiwa, M. Komatsu, S. Yoshida, A. Oshiro, G. Sugimoto, A. Mitsuyoshi, Y. Togashi, H. Kanaoka, A. Hattori, T. Higuchi, A. Nishimukai,** and **T. Wakai** declare no conflicts of interest.

Ethical approval

All procedures performed in this study 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 protocol was reviewed and approved by the Institutional Review Board of Hyogo College of Medicine (Approval Nos. 1969 and 106).

Informed consent

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

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Data statement

The datasets used in this study are available from the corresponding authors upon request.

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Figures

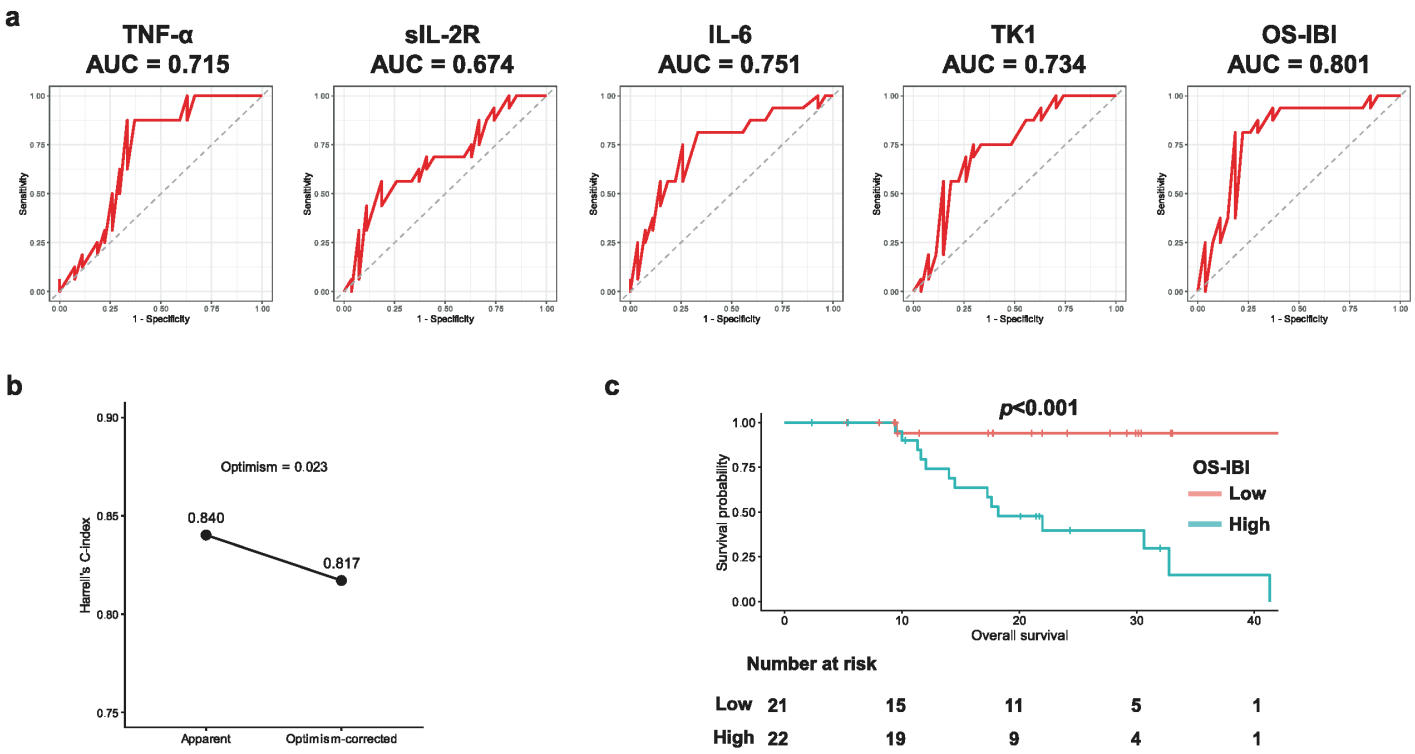


Figure 1

Discriminatory performance, internal validation, and prognostic significance of the Overall Survival Integrated Biomarker Index (OS-IBI). (a) Receiver operating characteristic (ROC) curves illustrating the discriminatory performance of individual biomarkers, including TNF-α, sIL-2R, IL-6, and TK1, and the combined OS-IBI.

(b) Internal validation of the OS-IBI using 1,000 bootstrap resamples. The apparent Harrell's concordance index (C-index) was 0.840, while the optimism-corrected C-index was 0.817, corresponding to a mean optimism of 0.023. **(c)** Kaplan–Meier curves comparing overall survival between patients with low and high OS-IBI values, stratified according to the median OS-IBI. AUC, area under the curve; TNF-α, tumor necrosis factor-α; sIL-2R, soluble interleukin-2 receptor; IL-6, interleukin-6; TK1, thymidine kinase 1; OS-IBI, Overall Survival Integrated Biomarker Index

	N (%)	HR (95% CI)	*p* value	P for Interaction
All	43 (100%)	1.62 (1.26–2.09)	<0.001	
Menopausal status				0.749
Premenopausal	6 (14%)	1.54 (0.62–3.83)	0.349	
Postmenopausal	37 (86%)	1.72 (1.26–2.34)	<0.001	
Recurrent vs. *de novo* advanced disease				0.452
Recurrence	23 (53%)	1.55 (1.12–2.14)	0.009	
Advanced	20 (47%)	2.17 (1.03–4.58)	0.043	
Treatment line				0.633
First line	24 (56%)	1.45 (1.05–2.01)	0.025	
≥2 lines	19 (44%)	1.78 (1.15–2.74)	0.010	
Estrogen receptor				0.194
ER-Positive	24 (56%)	5.84 (1.17–29.21)	0.032	
ER-Negative	19 (44%)	1.39 (0.99–1.95)	0.055	
HER2				0.101
HER2-Positive	12 (28%)	1.19 (0.79–1.81)	0.408	
HER2-Negative	30 (70%)	1.96 (1.32–2.91)	<0.001	
Drug class				0.499
Eribulin-based	23 (53%)	1.85 (1.18–2.90)	0.007	
Taxane-based	9 (21%)	1.24 (0.70–2.19)	0.461	
HER2-targeted antibody–drug conjugates	7 (16%)	1.57 (0.74–3.34)	0.240	
Other / trial	4 (9%)	Not estimable		

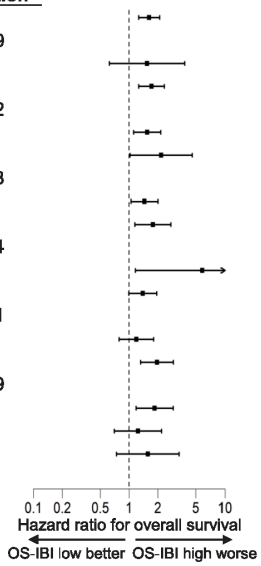


Figure 2

Forest plot showing hazard ratio (HR) and 95% confidence interval (CI) for overall survival (OS) per 1-unit increase in the Overall Survival Integrated Biomarker Index (OS-IBI) in the overall population and clinical subgroups. ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; ADC, antibody–drug conjugate

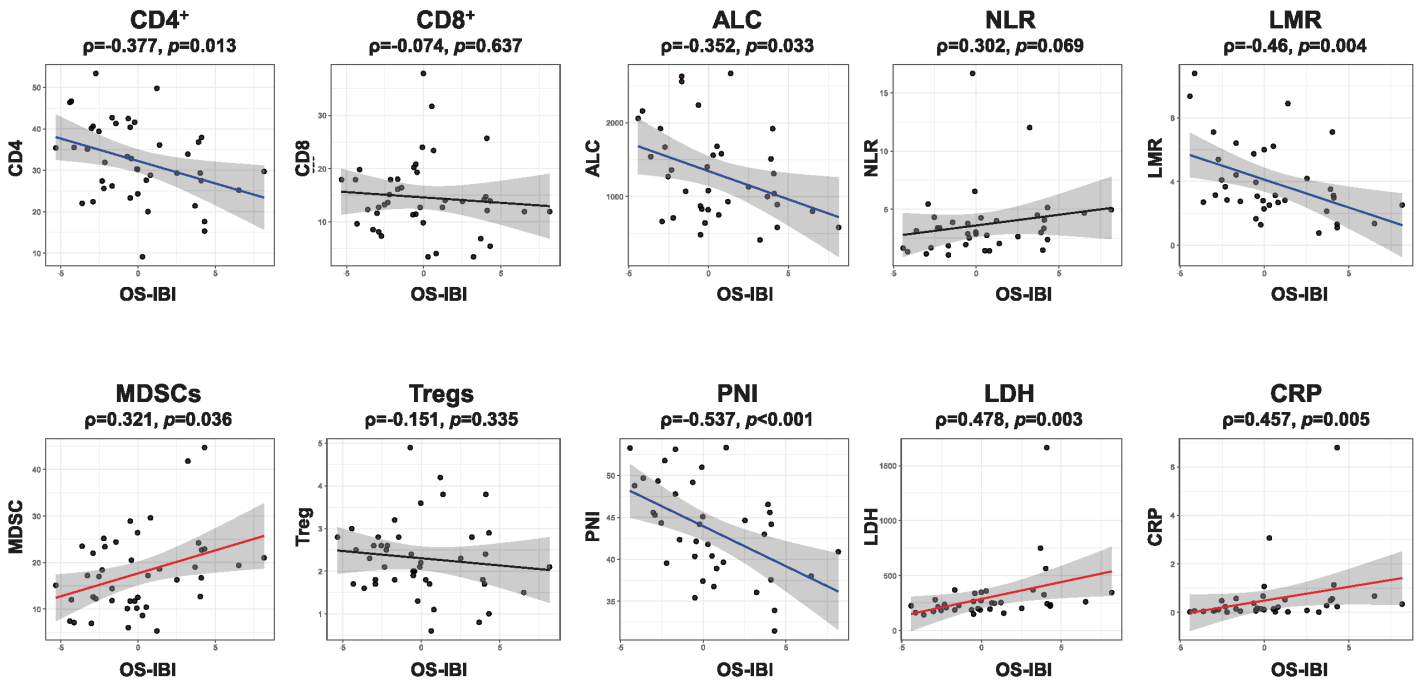


Figure 3

Scatter plots showing the association between the Overall Survival Integrated Biomarker Index (OS-IBI) and immune and inflammatory parameters. Spearman correlation coefficients (ρ) and p-values are shown. MDSCs, myeloid-derived suppressor cells; Tregs, regulatory T cells; ALC, absolute lymphocyte count; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PNI, prognostic nutritional index; LDH, lactate dehydrogenase; CRP, C-reactive protein

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure.pdf
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