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# Association of L-type amino acid transporter 1 (LAT1) with the immune system and prognosis in invasive breast cancer

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**Research Article**

**Keywords:** invasive breast cancer, L-type amino acid transporter 1, ER-positive and HER2-negative breast cancer, luminal B-like breast cancer

**Posted Date:** February 23rd, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-238356/v1

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# Abstract

**PURPOSE**: L-type amino acid transporter 1 (LAT1), also referred to as SLC7A5, is believed to regulate tumor metabolism and be associated with tumor proliferation. In invasive breast cancer, we clinicopathologically investigated the utility of LAT1 expression.

**METHODS**: LAT1 expression was evaluated via immunohistochemistry analyses in 250 breast cancer patients undergoing long-term follow-up. We assessed the relationship between LAT1 expression and the patients' outcomes and clinicopathological factors. Breast cancer-specific survival stratified by LAT1 expression was assessed.

**RESULTS**: High LAT1 expression was significantly correlated with estrogen receptor (ER) negativity, progesterone receptor negativity, high histological grade, increased tumor-infiltrating lymphocytes, and programmed death ligand 1 positivity. Among the ER-positive and human epidermal growth factor 2-negative type cases, high LAT1 was an independent indicator of poor outcomes (hazard ratio (HR) = 2.97; 95% confidence interval (CI), 1.16–7.62; $p$ = 0.023). Moreover, high LAT1 expression was an independent poor prognostic factor in luminal B-like breast cancer with aggressive features (HR = 3.39; 95% CI, 1.35–8.52; $p$ = 0.0094).

**CONCLUSIONS**: High LAT1 expression identified a subgroup of invasive breast cancer characterized by aggressive behavior and high tumor immunoreaction. Our findings suggest that LAT1 might be a candidate therapeutic target for breast cancer patients, particularly those with luminal B-like type breast cancer.


# Introduction

Survival of patients with breast cancer (BCa) has been improved by recent advances developments in treatment. However, approximately 20% of BCa patients have poor prognoses with recurrence and metastasis (Early Breast Cancer Trialists' Collaborative Group 2015). Characterizing the factors associated with tumor progression may lead to identifying the new molecular therapeutic targets. The uncontrolled proliferation altered metabolism and progression of BCa cells depends on uptake of sugars and amino acids (Lieu EL et al. 2020). Amino acids, including glutamine, are known to play a particularly important role in cell proliferation via the mTOR pathway (Mossmann D et al. 2018). The L-type amino acid transporter (LAT) enables the transport of large neutral essential amino acids into cells (Zhao Y et al. 2015; Pineda M et al. 1999). Interestingly LAT1, encoded by the *SLC7A5* gene, is generally overexpressed in malignant cells (Yanagida O et al. 2001; Kobayashi H et al. 2005; Nawashiro H et al. 2006; Nakanishi K et al. 2007; Kaira K et al. 2008a; Sakata T et al. 2009; Ichinoe M et al. 2011; Kaira K et al. 2012; Kaira K et al. 2013; Toyoda M et al. 2014). High LAT1 expression is closely related to the proliferation of tumor cells and angiogenesis in various types of cancer, such as melanoma (Shimizu A et al. 2015), lung cancer (Kaira K et al. 2019), pancreatic cancer (Altan B et al. 2018), and gastrointestinal cancer (Ohshima Y et al. 2016; Ogawa H et al. 2019). LAT1 overexpression is also associated with lymphovascular invasion,

lymphatic metastasis, and advanced stages of cancer (Yazawa T et al. 2015) and contributes to the development of therapeutic resistance in cancer cells.

The immune system affects all phases of tumor growth from initiation to progression and dissemination. Our previous studies confirmed that tumor immunity-associated biomarkers, such as tumor infiltrating lymphocytes (TILs) and programmed death ligand 1 (PD-L1), are related to the treatment-response and prognosis of BCa (Kurozumi S et al. 2019a, b). However, the role of LAT1 expression in breast tumor microenvironment, immunity and its prognostic significance remains undefined.

In this study, we evaluated the correlation between LAT1 protein expression and key clinicopathological factors of BCa patients and determined the relationship between LAT1 expression and patient's outcome.

# Materials And Methods

### Patient characteristics

This study has been ethically assessed by the Institutional Review Board of the Saitama Cancer Center (reference number 738). BCa patients (n=250) who underwent breast surgery at the Saitama Cancer Center were included in this study. None of the patient included in this study received neoadjuvant treatment. In total, 199 (79.6%) patients underwent breast-conserving surgery, and 119 (47.6%) underwent axillary lymph node dissection. Of the 250 patients, 48.4% had pathological T2–4 tumors, and 45.2% were pathological lymph node metastasis-positive cases.

Estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor 2 (HER2), and Ki-67 labeling index were assessed as detailed in our previous studies (Kurozumi S et al. 2017; Kurozumi S et al. 2018a). The ER positivity (≥1%) rate was 67.6%; 58.0% of the patients were PgR-positive (≥20%), and 17.2% were HER2-positive. The cohort was classified according to the intrinsic molecular subtypes (luminal A-like, Luminal B-like, HER2-positive, and triple negative types). The luminal A-like type was defined as "the patients who are PgR-positive and display low Ki67 staining (labeling index of ≤10%) in ER-positive and HER2-negative breast cancer" whereas other ER-positive and HER2-negative tumors were classified as luminal B-like type.

The density of stromal tumor-infiltrating lymphocytes (TILs) was evaluated according to the International Working Group guidelines (Salgado R et al. 2015). Cytoplasmic and/or membrane PD-L1 expression was assessed by immunohistochemistry (SP142; Roche, Switzerland; diluted 1:50), and the PD-L1 positivity cut-off value was determined as 1%. Details assessments of these biomarkers have been described in our previous paper (Kurozumi S et al. 2019b).

### LAT1 immunohistochemistry

LAT1 expression was assessed by immunohistochemistry using an affinity-purified polyclonal rabbit anti-human LAT1 antibody (Yanagida O et al. 2001) diluted to 1:5000. LAT1 protein expression was evaluated for cytoplasmic & membrane associated staining in full-face slides obtained from the 250 patients. For

clinicopathological and prognostic analyses the 250 samples were stratified into high- and low-LAT1 expression groups based on staining intensity.

The staining intensity of LAT1 expression on the cancer cells was scored as follows: 0 (no staining or staining of <10% of tumor cells), 1 (weak staining of ≥10% of tumor cells), 2 (moderate staining of ≥10% of tumor cells), and 3 (strong staining of ≥10% of tumor cells). In addition, tumors with a score of 2 or 3 were assigned to the high LAT1 expression group, whereas tumors with a score of 0 or 1 were placed in the low LAT1 expression group (Fig. 1 a-d: intensity score 0–3).

### Statistical analysis

The statistical analyses were undergone using SPSS statistical software v24.0 (IBM, Armonk, NY, USA). The relationships between LAT1 expression and several clinicopathological factors were evaluated using the chi-square and Fisher's exact tests. BCa-specific survival (BCSS) was used to evaluate the prognostic utility of LAT1 expression. For the univariate and multivariate prognostic assessments, the Cox proportional hazards regression model was used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs). The prognostic value of LAT1 mRNA (*SLC7A5)* expression was further evaluated using The Cancer Genome Atlas (TCGA) BRCA dataset as an external validation cohort. Briefly, the datasets of mRNA expression from RNASeqV2 were accessed along with de-identified clinical information for several clinicopathological factors and outcomes (Ciriello G et al. 2015; Kurozmi S et al. 2018b). The median value of *SLC7A5* expression was defined as a cut-off point.

## Results

### Association of LAT1 with clinicopathological factors

High LAT1 expression was present in 124 BCa patients (49.6%) whereas 126 patients (50.4%) had low LAT1 expression. Across the entire cohort, high LAT1 expression was significantly correlated with ER negativity ($p < 0.0001$), PgR negativity ($p < 0.0001$), HER2 positivity ($p < 0.0001$), large tumor size ($p = 0.016$), and high histological grade ($p < 0.0001$) (Table 1). In TCGA cohort, *SLC7A5* mRNA overexpression was associated with ER negativity ($p < 0.0001$), PgR negativity ($p < 0.0001$), large tumor size ($p = 0.047$), and high histological grade ($p < 0.0001$) (Supplementary Table 1).

For the relationships between LAT1 expression and tumor immunity-related biomarkers, high LAT1 expression was significantly associated with high TILs ($p < 0.0001$) and PD-L1 positivity ($p = 0.00024$) (Table 1). Twenty-five (20.2 %) cases with high LAT1 were included in high TILs group, and 18 (14.5 %) cases with high LAT1 had PD-L1 positivity. Survival curves stratified by LAT1/TILs and LAT1/PD-L1 levels were shown in the Supplementary Fig 1.

### Outcome analyses

The BCa specific survival (BCSS) differed significantly between the high and low LAT1 expression groups in the univariate analyses (HR = 1.97; 95% CI, 1.14–3.42; $p = 0.015$; Fig. 2a). Prognosis of high *SLC7A5*

mRNA expression group was a significant worse than that of low *SLC7A5* mRNA expression group in TCGA cohort (HR = 2.10; 95% CI, 1.34–3.29; $p$ = 0.0012; Fig. 2b).

### Prognostic analysis of *SLC7A5* mRNA based on ER status

To obtain insight into how LAT1 might be linked to survival, we evaluated the prognostic utility of *SLC7A5* mRNA expression according to ER status using TCGA database. Those with high *SLC7A5*-expressing tumors had a significantly lower BCSS than those with low *SLC7A5* tumors among patients with ER-positive cancer (HR = 2.13; 95% CI, 1.22–3.71; $p$ = 0.0075). By contrast, *SLC7A5* expression was not a prognostic factor among patients with ER-negative cancer (Supplementary Fig 2). At the protein level, high LAT1 expression was a significant prognostic marker in ER-positive type (HR = 2.22; 95% CI, 1.07–4.59; $p$ = 0.032), but not in ER-negative type (Supplementary Fig 2).

### Prognostic utility of LAT1 expression in ER-positive and HER2-negative BCa

In the 142 ER-positive and HER2-negative tumors, we investigated which factors were associated with LAT1 staining. The frequency of high LAT1 expression was 39.4% in luminal-like tumors. Among the luminal-like tumors, high LAT1 expression was significantly correlated with tumor size ($p$ = 0.018), high Ki67 labeling index ($p$ = 0.0017), and high histological grade ($p$ = 0.018) (Table 2). In the patients with luminal-like cancer, those with high LAT1-expressing tumors had significantly lower BCSS than those with low LAT1-expressing tumors (HR = 2.86; 95% CI, 1.26–6.48; $p$ = 0.012; Fig. 3a).

In addition to high LAT1 expression, univariate analysis showed that negative PgR expression (HR = 5.37; 95% CI, 2.12–13.63; $p$ = 0.00041) and positive nodal status (HR = 4.12; 95% CI, 1.64–10.57; $p$ = 0.0027) predicted reduced survival (Table 3). The multivariate analysis with a Cox proportional hazards regression model identified that LAT1 expression was a poor independent prognostic factor in patients with ER-positive and HER2-negative BCa (HR = 2.97; 95% CI, 1.16–7.62; $p$ = 0.023; Table 3). Moreover, among luminal B-like type cancer (the aggressive phenotype), high LAT1 expression was an independent poor prognostic factor for the univariate (HR = 2.58; 95% CI, 1.14–5.86; $p$ = 0.023; Fig. 3b) and multivariate (HR = 3.39; 95% CI, 1.35–8.52; $p$ = 0.0094; Supplementary Table 2) analyses.

## Discussion

The current study demonstrates that high LAT1 expression can be used to identify a subgroup of invasive BCa with aggressive behavior and high tumor immune-reaction (PD-L1 positivity and TILs upregulation). Some studies (Furuya M et al. 2012; El Ansari R et al. 2018) have suggested the prognostic utility of LAT1 in BCa. The present study indicated that high LAT1 expression was an independent poor prognostic factor in luminal B-like BCa. Androgen receptor (AR) signaling pathway plays an important role in BCa progression (Iacopetta D et al. 2012; Hickey TE et al. 2021). We have revealed that AR protein expression was highly expressed in approximately 55% of invasive BCa (Aleskandarany MA et al. 2016), and AR signaling pathway was related to treatment resistance and prognosis of BCa (Kurozumi S et al. 2019c). In the previous studies of prostate cancer, LAT function including LAT1 was regulated via AR signaling

pathway. And LAT1 expression was increased after hormone ablation in the metastatic prostate cancer (Wang Q et al. 2011; Wang Q et al. 2013). Furthermore, El Ansari et al. (2018) elucidated that high LAT1 expression levels are associated with high proliferation potential, as indicated by the high Nottingham Prognostic Index and Ki67 labeling index; high LAT1 expression levels are also a poor prognosis factor in luminal B-like type breast tumors. It has been postulated that luminal A-like and luminal B-like type tumors display significantly different proliferation abilities in ER-positive BCa (Kurozumi S et al. 2016; Burstein H J et al. 2019). Thus, a combinatorial chemotherapeutic approach is recommended because luminal B-like type tumors have an extremely high proliferation potential. It is difficult to suppress this proliferation by endocrine treatment alone. VEGF and Myc may be related to proliferation in luminal B-like BCa (Dadiani M et al. 2009). LAT1 contributes to angiogenesis in cancer in the presence of VEGF (Kaira K et al. 2008b). Moreover, Myc asserts its oncogenic functions partially through its control of LAT1 expression (Hayashi K et al. 2012). The results of this study suggest that the molecular pathways involving LAT1 is related to the proliferation or metastasis abilities of luminal B-like BCa. It may be possible to support the development of new drugs targeted against luminal B-like BCa by conducting a more detailed functional analysis of genes related to this molecular pathway.

Ansari RE et al. (2020) clarified that glutamine transporters, including LAT1, are associated with the expression of CD68-positive macrophages and PD1-positive lymphocytes in tumors. Moreover, they used triple-negative BCa (TNBC) cell lines to demonstrate that the inhibition of LAT1 reduced the expression of PD-L1. HIF1α is known to activate tumor-associated CD68-positive macrophages (Li N et al. 2016). Thus, LAT1 may be involved in the function of tumor-associated macrophages because it enhances the function of mTORC1 by controlling the HIF pathway (Land SC et al. 2007). The molecular pathway related to mTOR plays an essential role in the progression of BCa (Hare SH et al. 2017). Although an mTOR inhibitor is used to treat metastatic ER-positive BCa in clinical practice (Baselga J et al. 2012), its effectiveness in early-stage BCa remains to be elucidated. Recent investigations have observed an antitumor effect from using a LAT1 inhibitor because it suppresses the phosphoric acid of mTOR in tumor cells, inhibits its downstream cell proliferation signals, and elicits G1 arrest and apoptosis (Hayashi K et al. 2017). The mTOR pathway suppresses Treg cells and promotes the differentiation of CD8-positive T cells (Araki K et al. 2017; Sun IH et al. 2018). There are ongoing clinical trials for the combination of PI3K and PD-L1 inhibitors in TNBC (Page DB et al. 2019). To determine how LAT1 works in the antitumor immune reactions, additional functional studies will be necessary.

In conclusion, LAT1 expression was associated with immune-related biomarkers, such as TILs and PD-L1, and was strongly correlated with poorly differentiated tumors. These findings indicate that LAT1 may play important roles in antitumor immunity and promote BCa progression and metastasis, particularly in ER-positive and HER2-negative BCa. Further biological research regarding the ability of this new agent to inhibit LAT1 expression is warranted (Nawashiro H et al. 2006). Moreover, concomitant treatment using the LAT1 inhibitor and immune checkpoint inhibitors is expected to become an innovative therapeutic target in luminal B-like type.

# Declarations

### Acknowledgement

We gratefully acknowledge the work of our research technicians (Kei Masuda and Kumiko Sudo).

### Funding

This work was supported by Gunma University and International University of Health and Welfare.

### Compliance with ethical standards

Conflict of Interest

SK has received honoraria from Daiichi Sankyo co. ltd, Taiho Pharmaceutical co. ltd, Eli Lilly and Company, MSD K.K., AstraZeneca K.K., and Novartis Japan.

KK has received research grants and a speaker honorarium from Ono Pharmaceutical Company, Chugai Pharmaceutical, Taiho Pharmaceutical, and AstraZeneca.

TY has received research grants from Ono Pharmaceutical Co., Ltd., CHUGAI Pharmaceutical Co., Ltd., and Memolead CO.

KS has received research grants from CHUGAI Pharmaceutical Co., Ltd., and Ono Pharmaceutical Co., Ltd.

The other authors declare that they have no conflicts of interest.

Ethical Approval

This study was approved by the Saitama Cancer Center Institutional Review Board (reference number 738). All procedures performed in studies involving human participants were conducted in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed Consent

Informed consent was obtained from the participants included in this study.

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# Tables

**Table 1 Association of LAT1 expression with the clinicopathological factors in all patients.**

| Factors | | Expression of LAT1 | | | Significance |
|---|---|---|---|---|---|
| | | Low | High | Total | *p*-value |
| ER | Positive | 115 (68.0%) | 54 (32.0%) | 169 | < 0.0001 |
| | Negative | 11 (13.6%) | 70 (86.4%) | 81 | |
| PgR | Positive | 98 (67.6%) | 47 (32.4%) | 145 | < 0.0001 |
| | Negative | 28 (26.7%) | 77 (73.3%) | 105 | |
| HER2 | Positive | 9 (20.9%) | 34 (79.1%) | 43 | < 0.0001 |
| | Negative | 117 (56.5%) | 90 (43.5%) | 207 | |
| Tumor size | pT2-4 | 51 (42.1%) | 70 (57.9%) | 121 | 0.016 |
| | pT1 | 75 (58.1%) | 54 (41.9%) | 129 | |
| Nodal status | Positive | 55 (48.7%) | 58 (51.3%) | 113 | 0.70 |
| | Negative | 71 (51.8%) | 66 (48.2%) | 137 | |
| Histological grade | Grade 3 | 47 (32.4%) | 98 (67.6%) | 145 | < 0.0001 |
| | Grade 1, 2 | 79 (75.2%) | 26 (24.8%) | 105 | |
| TILs | High | 6 (19.4%) | 25 (80.6%) | 31 | < 0.0001 |
| | Intermediate | 14 (34.1%) | 27 (65.9%) | 41 | |
| | Low | 106 (59.6%) | 72 (40.4%) | 178 | |
| PD-L1 | Positive | 2 (10.0%) | 18 (90.0%) | 20 | 0.00024 |
| | Negative | 122 (53.5%) | 106 (46.5%) | 228 | |

Abbreviations: LAT1: L-type amino acid transporter 1, ER: estrogen receptor, PgR: progesterone receptor, HER2: human epidermal growth factor 2, TIL: tumor infiltrating lymphocytes.

**Table 2 Relationship between LAT1 expression and the clinicopathological factors in ER-positive/HER2-negative breast cancer**

| Factors | | Expression of LAT1 | | | Significance |
|---|---|---|---|---|---|
| | | Low | High | Total | *p*-value |
| Ki67 | ≥30% | 14 (46.7%) | 16 (53.3%) | 30 | 0.0017 |
| | >10 and <30% | 56 (70.9%) | 23 (29.1%) | 79 | |
| | ≤10% | 29 (87.9%) | 4 (12.1%) | 33 | |
| PgR | Positive | 64 (71.9%) | 25 (28.1%) | 89 | 0.57 |
| | Negative | 35 (66.0%) | 18 (34.0%) | 53 | |
| Tumor size | pT2-4 | 36 (59.0%) | 25 (41.0%) | 61 | 0.018 |
| | pT1 | 63 (77.8%) | 18 (22.2%) | 81 | |
| Nodal status | Positive | 46 (74.2%) | 16 (25.8%) | 62 | 0.36 |
| | Negative | 53 (66.3%) | 27 (33.8%) | 80 | |
| Histological grade | Grade 3 | 38 (59.4%) | 26 (40.6%) | 64 | 0.018 |
| | Grade 1, 2 | 61 (78.2%) | 17 (21.8%) | 78 | |
| Abbreviations: LAT1: L-type amino acid transporter 1, ER: estrogen receptor, PgR: progesterone receptor, HER2: human epidermal growth factor 2. | | | | | |

**Table 3 Survival analysis based on clinicopathological factors including protein expression of LAT1 in ER-positive/HER2-negative patients.**

| Factors | | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|---|
| | | Hazard Ratio | 95% CI | *p*-value | Hazard Ratio | 95% CI | *p*-value |
| LAT1 expression | Low | Reference | | | Reference | | |
| | High | 2.86 | 1.26–6.48 | 0.012 | 2.97 | 1.16–7.62 | 0.023 |
| Ki67 | <10% | Reference | | | Reference | | |
| | ≥10% | 3.26 | 0.76–13.90 | 0.11 | 2.01 | 0.41–9.76 | 0.39 |
| PgR | Positive | Reference | | | Reference | | |
| | Negative | 5.37 | 2.12–13.63 | 0.00041 | 4.62 | 1.80–11.82 | 0.0014 |
| Tumor size | pT1 | Reference | | | Reference | | |
| | pT2-4 | 1.80 | 0.79–4.10 | 0.16 | 0.89 | 0.36–2.24 | 0.81 |
| Nodal status | Negative | Reference | | | Reference | | |
| | Positive | 4.12 | 1.64–10.57 | 0.0027 | 4.32 | 1.58–11.79 | 0.0043 |
| Histological grade | Grade1-2 | Reference | | | Reference | | |
| | Grade 3 | 1.57 | 0.69–3.59 | 0.28 | 0.86 | 0.34–2.16 | 0.75 |

Abbreviations: LAT1: L-type amino acid transporter 1, ER: estrogen receptor, PgR: progesterone receptor, HER2: human epidermal growth factor 2.

# Supplementary Information

Supplemental Tables and Figures are not available with this version

# Figures

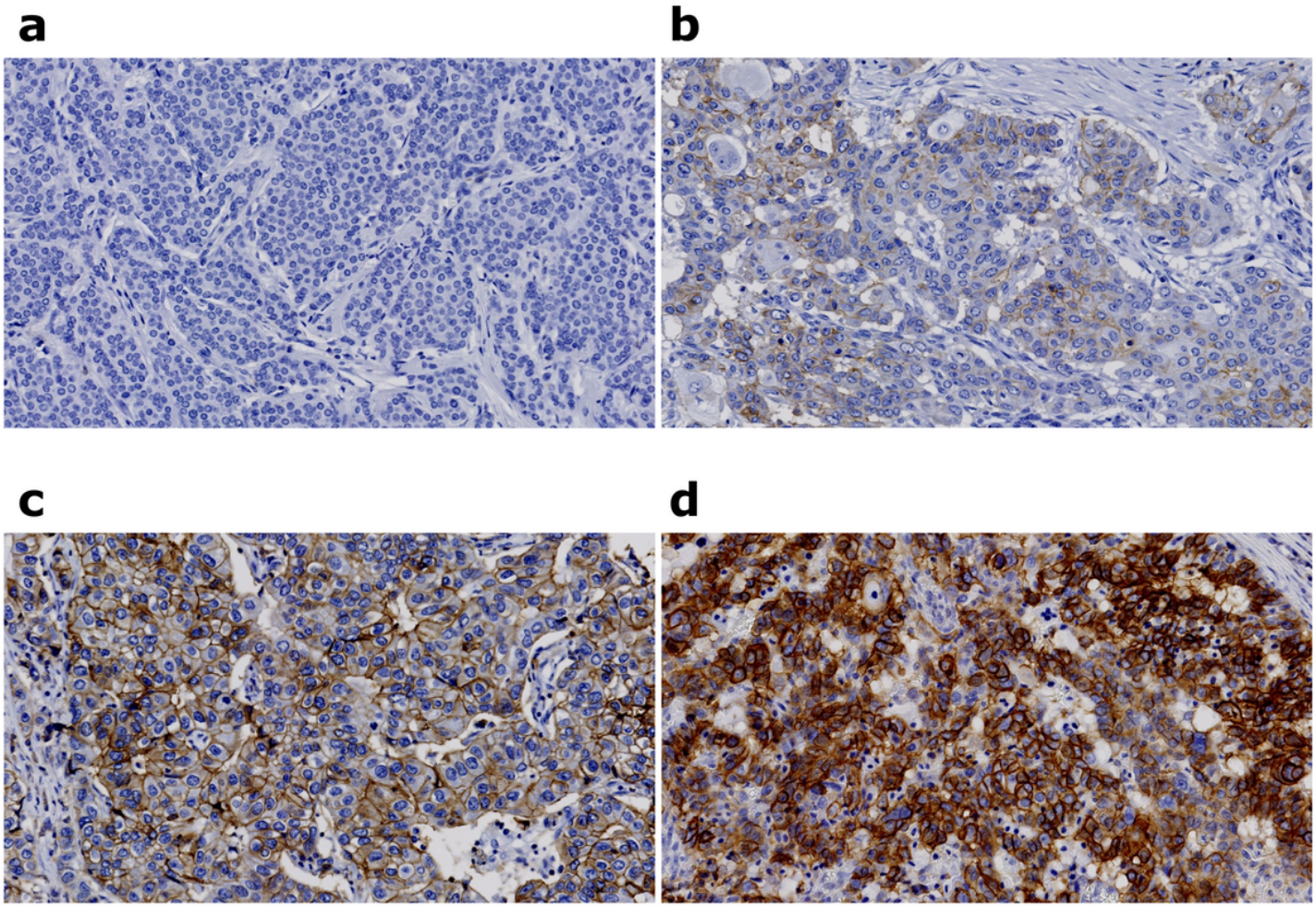


**Figure 1**

L-type amino acid transporter 1 (LAT1) expression in breast cancer immunohistochemical findings. (a) No staining (score 0), (b) weak staining (score 1), (c) moderate staining (score 2), and (d) strong staining (score 3) for LAT1 expression was detected in the cytoplasm of cancer cells

Image not available with this version

**Figure 2**

(a) Breast cancer-specific survival stratified by L-type amino acid transporter 1 (LAT1) protein expression, (b) Overall survival stratified by LAT1 mRNA expression

Image not available with this version

**Figure 3**

Breast cancer-specific survival stratified by L-type amino acid transporter 1 (LAT1) expression (a) in ER-positive and HER2-negative patients, and (b) in luminal B-like patients