

# Serum ATX as a novel biomarker for breast cancer

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## Abstract

Recent accumulating evidence indicates the biological actions of Autotaxin-Lysophosphatidic acid (ATX-LPA) signaling axis in malignant tumors. However, the role of Autotaxin-Lysophosphatidic acid signaling axis in breast cancer has not been reported. The present study aims to examine the alterations of serum autotaxin in breast cancer and discuss whether serum autotaxin could be useful as a novel parameter of breast cancer.

Serum autotaxin antigen was measured in 112 patients with breast cancer and 50 healthy volunteers by ELISA. The association of serum autotaxin antigen levels with clinicopathological parameters and outcomes of breast cancer was analyzed.

Serum autotaxin antigen was significantly higher in breast cancer patients than healthy volunteers ( $291.32 \pm 38.02$  ng/ml vs  $254.04 \pm 21.03$  ng/ml, respectively;  $P < .0001$ ). Serum autotaxin measurement successfully discriminated breast cancer patients from normal and healthy controls (AUC = 0.798, 95% CI: 0.732–0.864) with an optimal cut-off value of 267.34 ng/ml (sensitivity = 0.741, specificity = 0.800). Increased serum autotaxin was associated with breast cancer nodal status ( $P = .007$ ), Tumor-Node-Metastasis (TNM) stage ( $P = .009$ ) and Ki-67 index ( $P = .004$ ). Univariate and multivariate Cox regression analysis revealed that elevated serum autotaxin showed an independent prognostic value for poor Disease-free survival.

Our present study confirmed the elevation, potential diagnostic, and independent prognostic value of serum autotaxin for breast cancer. Serum autotaxin could serve as a reliable novel biomarker for breast cancer.

**Abbreviations:** ATX-LPA = autotaxin-Lysophosphatidic acid, AUC = area under the ROC curve, BCS = breast-conserving surgery, DFS = disease-free survival, ER = estrogen receptor, FISH = fluorescence in situ hybridization, Her-2 = human epidermal growth factor receptor 2, LPC = lysophosphatidylcholine, PR = progesterone receptor, ROC = receiver operating characteristics, TNBC = triple-negative breast cancer, TNM = tumor-node- metastasis.

**Keywords:** autotaxin, biomarker, breast cancer, prognosis

## 1. Introduction

Breast cancer is the most frequently occurring cancer in women and its incidence has been steadily increasing in China.<sup>[1,2]</sup> Despite the rising incidence of breast cancer, the survival rates have improved in recent years due to the deep research in biological behavior of breast cancer.<sup>[3,4]</sup> However, once treatment failure occurs the quality of life and the survival rate of patients is significantly affected. Therefore, it is essential to identify reliable prognostic factors to guide decision making during the treatment of breast cancer in order to improve prognosis.

Recent accumulating evidence indicates the biological actions of Autotaxin-Lysophosphatidic acid (ATX-LPA) signaling axis in cancer. The involvement of the lipid mediator Lysophosphatidic

acid (LPA) in regulating tumor progression, angiogenesis and metastasis has been increasingly recognized over the last decade. Much of the impetus for this started with the identification of autotaxin (ATX) as an extracellular lysophospholipase D, whose major signaling effect is to generate extracellular LPA.<sup>[5–7]</sup> Autotaxin (ATX) is a secreted 125 kDa glycoprotein and a member of nucleotide pyrophosphatase /phosphodiesterase family (NPP2). This enzyme was originally discovered as an autocrine motility stimulating factor released by human melanoma A-2058 cells.<sup>[8]</sup> Our understanding of ATX action increased significantly with the discovery of its lysophospholipase D (lysoPLD) activity, which can hydrolyze lysophosphatidylcholine (LPC) to lysophosphatidic acid (LPA).

The bioactivities of ATX can be primarily explained by the production of LPA, a bioactive lipid mediator. LPA acts through specific G protein-coupled receptors (GPCRs) to promote cellular proliferation, migration, and survival.<sup>[8]</sup> Cumulative evidence points to a role of LPA in cancer progression.<sup>[9,10]</sup> Increased ATX expression has been reported in various forms of cancer, such as glioblastoma, hepatocellular and thyroid carcinomas, breast, pancreatic and hematological cancers.<sup>[11,12]</sup> ATX expression was also reported higher in poorly differentiated tumors and, in independent studies, is correlated with invasiveness of cancer cells suggesting a higher metastatic potential of ATX-expressing tumors.<sup>[13]</sup>

In vitro and in vivo studies have demonstrated that increased ATX-LPA signaling contributes to cancer initiation and progression. However, few previous studies have investigated serum ATX expression in breast cancer. Therefore, in the current study, we investigated the association between serum ATX expression and biological behavior of breast cancer. This study was also designed to identify the prognostic value of serum ATX expression among patients with breast cancer.

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## 2. Materials and methods

### 2.1. Study population

From January 2010 to December 2010, serum ATX in a total of 112 patients who were treated for stage I–III invasive breast cancer at Henan Provincial People's Hospital were investigated. Inclusion criteria were: female; invasive breast cancer; underwent mastectomy or breast-conserving surgery; serum ATX levels were determined before surgery; appropriate adjuvant chemotherapy, adjuvant radiotherapy and endocrine therapy administered based on international guidelines. Exclusion criteria were: stage IV breast cancer; carcinoma in situ; neoadjuvant chemotherapy cases. This study was reported according to the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) criteria.<sup>[14]</sup> TNM staging was based on the sixth American Joint Committee on Cancer criteria. ER and PR positive were defined as tumors with >1% nuclear-stained cells. HER2-positivity was indicated by a 3+ or 2+ score from the immunohistochemical evaluation and was confirmed using a fluorescence in situ hybridization (FISH) test for HER2. A cut-off point of 14% was used for Ki-67 staining. The molecular subtypes were classified into four groups according to the molecular subtype consensus of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011.<sup>[15]</sup> The control group included 50 healthy volunteers from the Henan Provincial People's Hospital. None of the control patients had previously been diagnosed with any malignancy.

### 2.2. Sample collection and measurement of serum ATX

All serum samples were collected in the morning. Blood collected without anticoagulant was centrifuged at  $1600 \times g$  for 10 minutes at 4°C 1 hour after collection and transferred into tubes and kept at -80°C for further experimentation. Human serum ATX antigen was measured by a commercially available Human ENPP-2/Autotaxin Quantikine enzyme-linked immunosorbent assay (ELISA) Kit (R&D Systems Inc. DENP20, Minneapolis, MN), which contains Assay Diluent, Calibrator Diluent, Color Reagent A, Color Reagent B, Conjugate, Standard, Stop Solution and Wash Buffer Concentrate, and assayed using an automated immunoassay analyzer AIA-system (TOSOH Corp., Tokyo, Japan).

### 2.3. Follow-up

With the day pathological diagnosis was performed considered as the first day of follow-up, clinical follow-up was carried out every 6 to 12 months, which included recording patients' history, physical examination, chest radiography, breast and abdominal/pelvic ultrasonography, and bone scans. Disease-free survival (DFS) was defined to be from the time of surgery to the locoregional recurrence, distant metastasis, and death before recurrence. Locoregional recurrence was defined as pathologically confirmed relapse on the chest wall, supra- and infraclavicular fossa, axillary area, or internal mammary region. Distant metastasis was confirmed using medical imaging method, and pathology assessment if needed.

### 2.4. Ethics statement

This study was carried out in accordance with the ethical guidelines of the 1975 *Declaration of Helsinki* and was approved by the ethics committee of the Henan Provincial People's

Hospital. Written informed consent was obtained from every patient for the use of the pathological tissue samples and medical records for research purposes.

### 2.5. Statistical analysis

All data were expressed as the mean  $\pm$  standard deviation (SD). The statistical significance of differences between 2 groups was determined by Mann-Whitney *U* test and Kruskal-Wallis test was used to evaluate the statistical significance of differences of the variances for 3 or more groups. Receiver operating characteristics (ROC) analysis was applied to determine serum ATX sensitivity and specificity in discriminating between breast cancer and healthy control. Area under the ROC curve (AUC), sensitivity and specificity were used to assess the diagnostic power of serum ATX. The cut-off value was determined by the score closest to the value under peak sensitivity and specificity. DFS was estimated using the Kaplan-Meier method and compared using the log-rank test. Independent prognostic factors for DFS were identified by multivariate Cox proportional hazard analysis. All the statistical analyses were performed with SPSS 24 (SPSS, Chicago, IL) software. A *P* value < .05 was considered to be statistically significant.

## 3. Results

### 3.1. Patient and tumor characteristics

The study enrolled a total of 112 patients who were treated for stage I–III invasive breast cancer at Henan Provincial People's Hospital. The clinicopathological characteristics of the patients are shown in Table 1. The median age of the study population was 45 years (range 27–72 years), and 12.5% cases were young breast cancer. Stage I, II, and III breast cancers accounted for 5.4%, 60.7%, and 33.9% respectively. Among all the cases, Luminal A, Luminal B, HER2-positive and triple-negative subtype breast cancer accounted for 17.9%, 34.8%, 20.5%, and 26.8%, respectively. Most cases (80.4%) received anthracycline and taxane based chemotherapy, other patients received anthracycline or taxane based chemotherapy. Thirteen patients (11.6%) received breast-conserving surgery, 99 patients (88.4%) received a mastectomy operation.

### 3.2. Relationship between serum ATX and clinicopathological characteristics of patients

Serum ATX levels were measured in 112 breast cancer patients and 50 healthy volunteers. The serum ATX level in breast cancer patients was significantly higher than in normal controls ( $291.32 \pm 38.02$  ng/ml vs  $254.04 \pm 21.03$  ng/ml, respectively;  $P < .0001$ ). Furthermore, the correlation between serum ATX levels and clinicopathological characteristics of breast patients was analyzed, which was shown in Table 1. Serum ATX levels were correlated with the nodal status ( $P = .007$ ), TNM stage ( $P = .009$ ) and Ki-67 index ( $P = .004$ ) of breast cancer patients. Advanced axillary lymph nodal and TNM stage, higher Ki-67 index breast cancer patients exhibited higher proportion of elevated serum ATX levels. Serum ATX levels were not associated with age, tumor size, ER, PR, Her-2, molecular subtype and chemotherapy type.

### 3.3. Diagnostic value of serum ATX in BC patients

In order to verify whether the serum ATX could be used as a new diagnostic marker for breast cancer, ROC analysis was

**Table 1**  
**Clinicopathological characteristic of subjects and correlation between serum ATX and clinicopathological factors.**

Characteristics	n (%)	Serum ATX level	P value
Age			.799
≤35 y	14 (12.5%)	290.57 ± 27.85	
>35 y	98 (87.5%)	291.42 ± 39.38	
Tumor size			.275
T1	29 (25.9%)	287.62 ± 40.23	
T2	74 (66.1%)	294.21 ± 38.94	
T3	9 (8.0%)	279.46 ± 16.65	
Nodal status			.007
N0	43 (38.4%)	282.83 ± 35.04	
N1	33 (29.5%)	285.75 ± 42.01	
N2	15 (13.4%)	298.64 ± 32.84	
N3	21 (18.7%)	312.19 ± 34.11	
TNM stage			.009
I	6 (5.4%)	287.59 ± 20.63	
II	68 (60.7%)	284.03 ± 39.57	
III	38 (33.9%)	304.94 ± 33.98	
ER			.133
Negative	42 (37.5%)	298.03 ± 36.60	
Positive	70 (62.5%)	287.29 ± 38.55	
PR			.101
Negative	46 (41.1%)	298.10 ± 34.93	
Positive	66 (58.9%)	286.59 ± 39.61	
Her-2			.080
Negative	84 (75.0%)	303.04 ± 32.93	
Positive	28 (25.0%)	287.41 ± 49.20	
Ki-67 (%)			.004
<14%	24 (21.4%)	272.68 ± 40.33	
≥14	88 (78.6%)	298.45 ± 34.79	
Molecular subtype			.806
Luminal A	20 (17.9%)	297.38 ± 25.55	
Luminal B	39 (34.8%)	288.63 ± 42.54	
Her-2 positive	23 (20.5%)	289.11 ± 42.83	
TNBC	30 (26.8%)	292.46 ± 36.10	
Chemotherapy type			.871
Anthracycline/taxane-based	90 (80.4%)	291.78 ± 39.39	
Anthracycline based	13 (11.6%)	291.51 ± 37.30	
Taxane based	9 (8.0%)	286.38 ± 26.16	
Surgery type			.716
BCS	13 (11.6%)	290.54 ± 46.24	
Mastectomy	99 (88.4%)	291.42 ± 37.09	

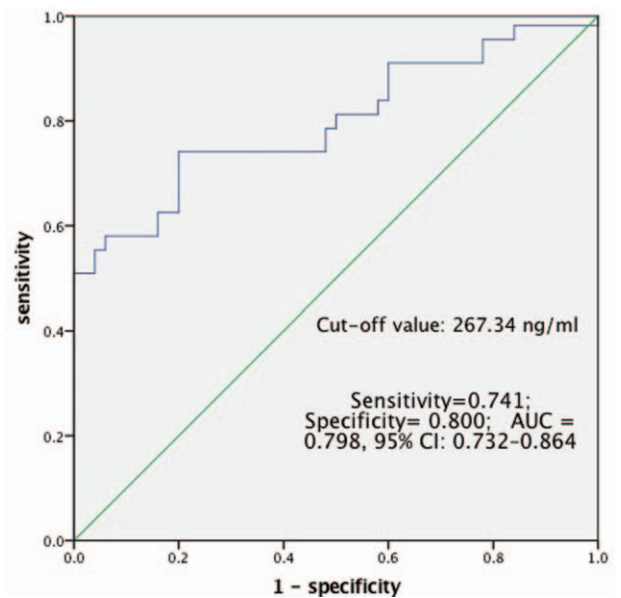
BCS=breast-conserving surgery, ER=estrogen receptor, Her-2=human epidermal growth factor receptor 2, PR=progesterone receptor, TNBC=triple-negative breast cancer, TNM=tumor-node-metastasis.

\*P<.05 indicates a significant difference.

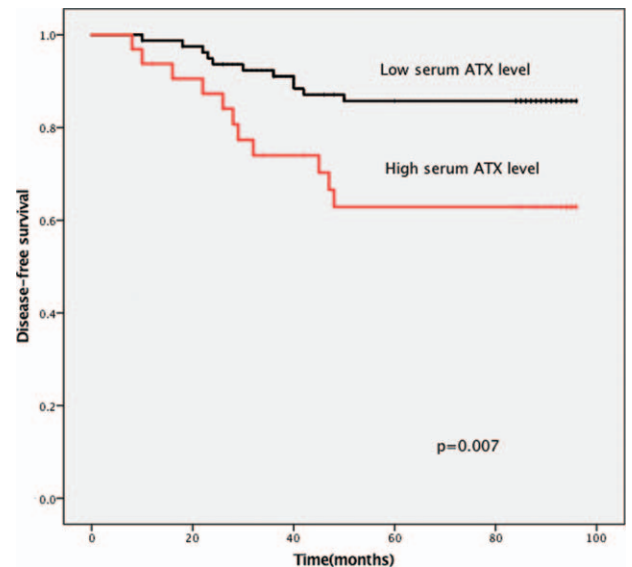
conducted. Serum ATX successfully discriminated BC patients from healthy controls (AUC=0.798, 95% CI: 0.732–0.864), and serum ATX displayed better discriminative ability (sensitivity, 0.741; specificity, 0.800) at the optimal cut-off value of 267.34 ng/ml (Fig. 1).

**3.4. Prognostic value of serum ATX levels in breast cancer patients**

Serum ATX levels were effective BC prognostic indicators as shown by ROC analysis and an optimal cut-off value was determined at 312.06ng/ml. Our breast cancer cohort was divided into 2 groups, high serum ATX group (serum ATX > 312.06ng/ml, n=32), low serum ATX group (serum ATX < 312.06ng/ml, n=80). Elevated serum ATX levels were significantly associated with poor DFS of breast cancer patients (Fig. 2).



**Figure 1.** ROC analyses for serum ATX to differentiate breast cancers from healthy controls.



**Figure 2.** Kaplan-Meier survival curve of patients with high and low serum ATX levels.

Univariate analysis revealed tumor size, nodal status, stage and serum ATX levels were associated with DFS of breast cancer patients. Multivariate Cox regression analysis according to tumor size, nodal status, stage and serum ATX levels revealed that elevated serum ATX level was independent prognostic factor for DFS (Table 2).

**4. Discussion**

The ATX-LPA signaling axis acts on a series of G protein coupled receptors (GPCRs), leading to diverse biological actions, including breast cancer tumorigenesis. Compared with normal mammary epithelial cells, ATX activity is significantly increased

**Table 2****Multivariate Cox analysis of variables considered for disease-free survival rates of breast cancer patients.**

Variables	HR	95% CI	P value
Tumor size			
T1-T2			
T3	1.358	0.541–3.408	.514
Nodal status			
Negative			
Positive	4.625	1.559–13.723	.006
TNM stage			
I-II			
III	1.777	0.701–4.503	.226
ATX level			
>267.34 ng/ml			
≤267.34 ng/ml	2.674	1.043–6.861	.041

ATX = autotaxin, TNM = tumor-node-metastasis.

\* $P < .05$  indicates a significant difference.

in breast cancer cells.<sup>[16]</sup> Animal model experiments have confirmed that transduction of ATX or LPA receptors (LPA1, LPA2, LPA3) can lead to the development of breast slow inflammatory disease, intraepithelial neoplasia of breast cells and invasive breast cancer.<sup>[17]</sup> Our present study confirmed previous studies reported, the serum ATX level in breast cancer patients was significantly higher than in normal controls ( $291.32 \pm 38.02$  ng/ml vs  $254.04 \pm 21.03$  ng/ml, respectively;  $P < .0001$ ). This suggests that the ATX-LPA signaling pathway is another important mechanism for breast cancer tumorigenesis.

Our ROC analysis results suggest that serum ATX can distinguish BC patients from healthy controls (AUC = 0.798, 95% CI: 0.732–0.864), at the optimal cut-off value of 267.34 ng/ml, serum ATX displayed better discriminative ability (sensitivity, 0.741; specificity, 0.800). Serum ATX becomes a new potential diagnostic marker for breast cancer.

Among the 40 genes identified by high-metastasis breast cancer gene chip analysis, ATX is one of them. ATX has been shown to promote tumor metastasis before ATX's phospholipase D (PLD) activity is discovered. ATX promotes the metastasis of breast cancer through LPA. ATX can promote the proliferation of vascular endothelial cells, and LPA can stimulate the synthesis and release of vascular endothelial growth factor (VEGF) and thus promote angiogenesis. In addition, LPA could also promote breast cancer cell migration by activating PI3K/PAK1/ ERK signaling pathway.<sup>[18]</sup> Another study also confirmed that Lysophosphatidic acid regulates the motility of MCF10CA1a breast cancer cell.<sup>[19]</sup> Our present study also confirmed that advanced axillary lymph nodal and TNM stage, higher Ki-67 index breast cancer patients exhibited higher proportion of elevated serum ATX levels. We hypothesized that ATX is involved in the invasion and progression of breast cancer.

Indeed, aberrant expression of ATX and LPA receptors occurs during the development and progression of breast cancer, and thus targeting ATX-LPA signaling pathway provides new ideas for breast cancer treatment. Many studies are in progress, and some researches have made gratifying achievements.<sup>[20,21]</sup>

A large number of evidence indicate that ATX-LPA is associated with chemotherapy resistance of cancer, and in breast cancer, ATX can reverse paclitaxel-induced cell apoptosis.<sup>[22]</sup> In the MCF-7 breast cancer cell line, the expression of LPA receptor is increased, which promotes cell proliferation and reverses tamoxifen-induced cell cycle arrest and apoptosis.<sup>[23]</sup> ATX is

associated with breast cancer chemotherapy, endocrine therapy response, and aberrant ATX overexpression may influence the effect of breast cancer treatment. Our present study confirmed the independent prognostic value of elevated serum ATX levels for breast cancer, elevated ATX levels were accompanied by worse DFS. Serum ATX provides additional valuable prognostic information for breast cancer.

In conclusion, our present study confirmed the elevation of serum ATX in breast cancer, and serum ATX becomes a new potential diagnostic and prognostic circulating marker for breast cancer. Further analyses, including prospective studies, are needed to verify the value of this serum circulating biomarkers in treatment decision-making area for breast cancer.

## Author contributions

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**Writing – review & editing:** Yingbo Shao, Hui Liu.

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