RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ
Journal of Advanced Research in Health Sciences /
Sağlık Bilimlerinde İleri Araştırmalar Dergisi 2025
DOI: 10.26650/JARHS2025-1606937

CAN LYVE-1 MOLECULE BE A DIAGNOSTIC BIOMARKER IN PATIENTS WITH ADVANCED LUNG CANCER?

LYVE-1 MOLEKÜLÜ İLERİ EVRE AKCİĞER KANSERLİ HASTALARDA TANISAL BİR BİYOBELİRTEÇ OLABİLİR Mİ?

Murat SERİLMEZ[1], Emre ÖZGÜR[1], Hilal OĞUZ SOYDİNÇ[1], Ceren TİLGEN YASASEVER[1], Faruk TAŞ[2], Uğur GEZER[1], Derya DURANYILDIZ[1]

[1]İstanbul University, Oncology Institute, Department of Basic Oncology, İstanbul, Türkiye
[2]İstanbul University, Oncology Institute, Department of Clinic Oncology, İstanbul, Türkiye

ORCID ID: M.S. 0000-0001-8502-2505; E.Ö. 0000-0002-4995-4759; H.O.S. 0000-0002-7858-1584; C.T.Y. 0000-0002-8732-4117; F.T. 0000-0001-6514-7116; U.G. 0000-0001-8471-5254; H.D.D. 0000-0002-2820-3227

Citation/Atıf: Serilmez M, Özgür E, Oğuz Soydinç H, Tilgen Yasasever C, Taş F, Gezer U, et al. Can LYVE-1 molecule be a diagnostic biomarker in patients with advanced lung cancer? Journal of Advanced Research in Health Sciences 2025;8(1):7-14. https://doi.org/10.26650/JARHS2025-1606937

ABSTRACT

Objective: Primary lung cancers that originate from epithelial cells are classified as carcinomas. Close monitoring of patients with predisposing conditions can enhance early diagnosis rates and facilitate the implementation of therapeutic approaches. LYVE-1 protein is localized to the lymphatic endothelial layer. This study aimed to evaluate the clinical utility of the serum protein and circulating mRNA of LYVE-1 in lung cancer.

Materials and Methods: We performed ELISA and Real-time PCR to measure and compare the serum protein and circulating mRNA levels of LYVE-1 in the peripheral blood of 60 patients with advanced lung cancer and 20 controls.

Results: Serum LYVE-1 protein and gene expression levels were significantly higher in patients with lung cancer than in controls (p=0.001). There was no association between LYVE-1 (both protein and gene) clinical parameters. The outcome of the ROC analysis, serum LYVE-1 protein (AUC: 0.873) or *LYVE-1* gene (AUC: 0.921) in lung cancer patients.

Conclusion: As far as we are aware, this study represents the first investigation to compare the protein and mRNA levels of *LYVE-1* in the blood samples of lung cancer patients. Additional research involving a larger cohort of subjects will be necessary to gain a deeper understanding of the mechanisms and consequences of LYVE-1 inhibitors in lung cancer.

Keywords: LYVE-1, lung cancer, lymphatic biomarker

ÖZ

Amaç: Primer akciğer kanserleri epitelyal hücrelerden türeyen karsinomlardır. Predispozan bozukluğu olan hastaların yakın takibi, erken tanı ve küratif tedavi yöntemlerinin oranlarında artış sağlayabilir. Hyaluronan için bir reseptör molekülü olan LYVE-1, lenfatik endotelde ifade edilir. Bu çalışma, akciğer kanserinde LYVE-1'in serum proteini ve dolaşımdaki mRNA'sının klinik faydasını değerlendirmeyi amaçlamıştır.

Gereç ve Yöntemler: İleri evre 60 akciğer kanseri hastası ve 20 sağlıklı kontrolün periferik kanında LYVE-1'in serum protein ve dolaşımdaki mRNA seviyelerini ölçmek ve karşılaştırmak için ELISA ve Gerçek Zamanlı PCR uygulaması gerçekleştirildi.

Bulgular: Akciğer kanserli hastalarda serum LYVE-1 protein ve gen ekspresyon düzeyleri kontrol grubuna göre anlamlı derecede yüksek bulunmuştur (p=0,001). Akciğer kanseri hastalarında serum LYVE-1 protein ve gen ekspresyon düzeyleri ile klinik parametreler arasında bir ilişki bulunmamıştır. ROC analizine göre eğri altında kalan alanlar hesaplandığında; serum LYVE-1 proteini ve geni için AUC (Area Under Curve) değerleri sırasıyla 0,873, 0,921 şeklinde bulunmuştur.

Sonuç: Bu çalışma, akciğer kanseri hastalarının kan örneklerinde LYVE-1'in protein ve mRNA düzeylerini karşılaştıran ilk çalışmadır. Akciğer kanserinde LYVE-1 inhibitörlerinin mekanizmalarını ve sonuçlarını daha derinlemesine anlamak için daha geniş bir denek grubunu içeren ek araştırmalara ihtiyaç duyulmaktadır. Bu tür araştırmalar, bu alana ilişkin değerli bilgiler sağlamada faydalı olacaktır.

Anahtar kelimeler: LYVE-1, akciğer kanseri, lenfatik biyomarker

Corresponding Author/Sorumlu Yazar: Murat SERİLMEZ **E-mail:** murat.serilmez@istanbul.edu.tr

Submitted/Başvuru: 25.12.2024 • **Revision Requested/Revizyon Talebi:** 09.01.2025 • **Last Revision Received/Son Revizyon:** 07.02.2025 • **Accepted/Kabul:** 10.02.2025 • **Published Online/Online Yayın:** 20.02.2025

INTRODUCTION

Lung cancer is the primary cause of cancer-related deaths globally, with a 5-year survival rate of 18%. As traditional therapies are largely insufficient for curative intent, the early diagnosis and identification of molecular targets are being investigated. The identification of diagnostic, prognostic, and predictive markers is crucial in clinical practice because lung cancer represents a heterogeneous group of diseases with diverse biological and clinical characteristics. A new hot topic to debate is finding non-invasive techniques, such as circulating biomarkers, to increase the rate of early diagnosis and targets for curative treatment modalities in lung cancer (1). The molecular mechanisms underlying lung cancer and its metastasis require further investigation because the disease is often metastatic at the time of diagnosis (2).

The lymphatic capillaries are composed of a single layer of lymphatic endothelial cells (LECs). In contrast to the anatomical structure of the blood vessels, the endothelium of the lymphatic system is surrounded by a muscle layer, pericytes, and a continuous basement membrane. Owing to this function, lymph node outflow is avoided (3). In addition, the endothelial cells of the lymphatic system are covered with elastic filaments that adhere to the extracellular matrix. Therefore, intercellular pressure is prevented during the collapse of the lymphatic capillary tissues due to the transformation of the lymphatic capillaries. As the pressure increases, the filaments elongate and overlap the lymphatic openings of the endothelial cells, allowing the lymphatic fluid, macromolecules, and cells to enter, which causes the capillaries to open. Lymph node metastasis, classification, prognosis, and treatment of malignant tumours are among the most important factors in determining the form. Although the clinical features of metastasis are known, their molecular mechanisms are limited, and LECs are pivotal in the development and maintenance of the lymphatic system. Moreover, the vascular system plays a fundamental role in regulating immune responses. Studying lymphatic endothelial cells (LECs) in both *in vivo* and *in vitro* models is often challenging, as these models can only partially replicate their behaviour and phenotypes. It has been demonstrated that LECs gradually lose their lymphatic markers over time, highlighting their adaptability and diversity in vivo. Given that LECs uniquely express LYVE-1, coating them with hyaluronic acid (HA) has been found to preserve their phenotypes and functions (4).

Most cancers, especially carcinomas, metastasize to the lymph centres from which they drain. Systemic spread can be stopped by attacking the higher endothelial vessels (HEV) in the paracortex (5, 6). Be that because it may, at show, the components by which tumours assault and move interior the lymphatics are not totally caught on to get to lymphatic capillaries and action to exhausting lymph centre points in the midst of secure observation, safe adjust, and assurance of disturbance (7, 8). These consolidate chemotaxis, haptotaxis, and heading by means of haptotaxis aerotaxis, which connects to the vascular surface through receptors such as integrins and MMPs (cross section metalloproteinases) and ADAMs (A Disintegrin and Metalloproteinases) (9-11). This brief review may be a new the coming approximately instrument for lymphatic segment counting of the broad polysaccharide HA and its key lymphatic structure and safe cell receptors LYVE-1 and CD44, respectively, that some tumours can also use this centre to help with nodal metastasis.

Most HA-binding proteins belong to the superfamily of Link proteins, all of which share a shared hyaluronan-binding domain called the “Link” module. LYVE-1 was characterised in the pooled Human Genome Sciences/TIGR EST database (12,13).

LYVE-1 exhibits a molecular mass of 60-70 kDa, A distinguishing feature of LYVE-1 is its uniqueness, as it is not found in its closest homologous receptors, such as CD44. Similar to CD44, the most closely related receptor to LYVE-1, it was bound -residue hydrophobic domain containing a cysteine residue (14).

Genetic changes in the *LYVE-1* gene play an important role in patients with lung cancer. Alterations in the expression of this gene contribute to a more aggressive cancer phenotype. However, how exactly do changes in the *LYVE-1* gene influence cancer development? Our goal is to provide valuable insights for clinicians in guiding treatment decisions and slowing or halting cancer progression. We believe that this study will make a crucial contribution to the field. In our research, we found elevated levels of LYVE-1 protein in the serum of lung cancer patients, which can be easily obtained. These findings indicate that LYVE-1 holds potential as a biomarker for lung cancer, as its protein levels can be evaluated in patients to support diagnosis or prognosis. We hope that our findings will guide diagnostic approaches. Additionally, no similar studies were found in the literature comparing both protein and mRNA levels of LYVE-1 in lung cancer serum, making our study a valuable addition to the existing body of research.

MATERIAL AND METHODS

This study investigated 60 lung cancer patients and 20 control cohorts that were collected between 2015 and 2016. Of the 20 individuals in the healthy control group, 15 were male, 10 were over the age of 60, and 12 were smokers. Serum protein and circulating LYVE-1 levels were measured using ELISA in the two cohorts. Our study on human materials was approved by the İstanbul University Ethics Committee (Date: 27.08.2014, No: 1311). This study was conducted according to the Declaration of Helsinki (1989), and all the volunteers were informed about this study’s content.

Blood samples were collected from both patients and healthy controls via venipuncture, ensuring that the blood clotted at room temperature. Serum preparation involved centrifugation at 4000 rpm for 10 min at room temperature. For storage, the initial admission samples were frozen immediately at -80°C prior to treatment, while the follow-up samples were frozen at -20°C. Patient classification was performed on the basis of disease staging using the AJCC and IUCC systems, complemen-

Table 1: Primer sequences

Gene Name	Forward primer F (5′-3′)	Reverse primer R (3′-5′)
LYVE-1 NM_016164	TCCTATCCTCCTACCTCCAAAG	CGTATCCTCAGCCTTGTTCTATT
GAPDH NM_002046	GCTCTCTGCTCCTCCTGTTC	ACGACCAATCCGTTGACTC

ted by a comprehensive clinical evaluation, which included detailed medical history, physical examination, and blood tests. Eligibility for treatment was determined using specific criteria, including a performance status of ECOG ≤ 2, an absolute neutrophil count exceeding 1500/µL, and a platelet count greater than 100,000/µL. A multidisciplinary approach was employed for treatment planning to ensure comprehensive care.

Determination of protein levels of the LYVE-1 molecule

An ELISA kit ((Catalog no: CSB-EL013282HU, Shanghai Yehua Biological Technology Shanghai, China) was used to measure the serum LYVE-1 level. A volume of 40 µL of serum samples and 10 µL of LYVE-1 antibody were added to all antibody-coated wells using an automated pipette. Additionally, 50 µL of the prepared standards were introduced into the designated wells. Subsequently, 50 µL of the streptavidin-HRP solution was added to each well. The microplates were incubated at 37°C for 1 h to facilitate the formation of the antibody-antigen-antibody complex. Following the incubation, the wells were washed five times with 300 µL of washing solution and thoroughly dried. Next, 50 µL of Chromogen Reagent A was added, followed by 50 µL of Chromogen Reagent B, and the plate was incubated at 37°C for 10 min to allow for colour development. The enzymatic reaction was terminated by the addition of 50 µL of the stop solution. The absorbance values of the samples were measured at 450 nm using an ELISA reader (ChroMate 4300 Microplate Reader, USA).

The concentrations of the samples were determined using a standard curve generated from the absorbance values of known standards. These calculated concentrations were automatically compared with the experimentally measured concentrations.

Determination of the Gene Expression Levels RNA isolation
A volume of 200 µL of serum was transferred into 1.5 mL Eppendorf tubes, followed by the addition of 800 µL of TRIzol reagent. The mixture was homogenised and incubated at room temperature for 5 min. Subsequently, 200 µL of chloroform was added to the tubes, and the mixture was incubated at room temperature for another 5 min. The samples were centrifuged at 12,000×g for 15 min, resulting in phase separation. The upper aqueous phase was carefully transferred to new 1.5 mL Eppendorf tubes, and 550 µL of isopropanol was added. The mixture was incubated at room temperature for 5–10 min and centrifuged again at 12,000×g for 10 min. The supernatant was discarded, and the resulting pellet was washed with 1 mL of 75% cold ethanol, followed by cortexin. The sample was centrifuged at 7,500×g for 5 min, and the supernatant was discarded. The RNA pellet obtained was dissolved in RNase-free water.

Complementary DNA (cDNA) synthesis
Complementary DNA (cDNA) synthesis was performed from total RNA using a commercially available kit. For this process, 6 µL of total RNA, 0.5 µL of random hexamer primers, and 6 µL of dH2O were added to 0.5 mL PCR tubes. were sequentially mixed and placed in a conventional PCR device. The resulting cDNA samples were stored at -20°C for subsequent use.

Real-Time Polymerase Chain Reaction (RT-PCR)
The expression levels of *LYVE-1* molecules were analysed by Real-Time PCR on The LightCycler® 480 System (F. Hoffmann-La Roche Ltd) using the SYBR GreenMaster PCR Kit (Jena Bioscience GmbH Dortmund Germany). mRNA expression normalisation was performed using *GADPH*. RT-PCR conditions were selected according to the GreenMaster PCR protocol. The primers listed in Table 1 were used.

Following the RT-PCR position, "melting curve analysis" was performed. The aim of this is to confirm whether the RT-PCR products formed are the desired true products and to exclude non-specific products and secondary structures such as primer dimers. This is done by analysing the melting temperature (Tm) of the RT-PCR products. For this purpose, the samples were subjected to an increasing temperature rise of 0.2°C per second from 55°C to 95°C. In this process, melting curves in the range of approx. 75-80°C occur, depending on the composition of the RT-PCR product.

In the RT-PCR reaction, the cycle at which the fluorescence level exceeds the threshold value that can be measured by the instrument is called Ct (cycle treshold). According to the Ct value obtained, the gene expressions were calculated and evaluated using the $2^{-\Delta\Delta Ct}$ method.

$$\Delta\Delta Ct = (Ct_{target} - Ct_{referance})_{sample} – (Ct_{target} - Ct_{referance})_{control}$$

$$2^{-\Delta\Delta Ct} = 2^{-[(Cttarget - Ctreferance)sample – (Cttarget - Ctreferance)control}$$

Statistical analysis
The clinical parameters of the study groups and expression results were analyzed using SPSS version 30. Continuous variables were classified using median values as a cut-off point. The expression levels was evaluated by using Kolmogorov-Smirnov test. To compare the patient and healthy groups, the Wilcoxon rank test was applied to target LYVE-1 protein and gene expression levels. Spearman-rho correlation analysis was used to assess the relationships between the LYVE-1 protein and gene. ROC curve analysis was performed for evaluation. The Mann–Whitney U test was used to examine the effect of the clinical parameters LYVE-1 levels. Assuming an independent two-sample t-test based on the description. We calculated the standardised effect size and power range using the Power G program. This means the study is well-powered to detect a statistically signi-

Table 2: Arithmetic mean (x), standard deviation (sd), median (m), minimum (min) and maximum values (max) of protein levels in the lung cancer patients and healthy control groups

		LYVE1 protein (ng/ml)				
		Mean (x)	Standard Deviation (sd)	Median (m)	Minimum (min)	Maximum (max)
Group	Patient	22.44	18.92	10.80	6.20	62.90
	Control	7.26	1.92	7.70	2.70	10.50

Table 3: Arithmetic mean (x), standard deviation (sd), median (m), minimum (min) and maximum values (max) of gene expressions in the lung cancer patients and healthy control groups

		LYVE1 gene expression				
		Mean (x)	Standard Deviation (sd)	Median (m)	Minimum (min)	Maximum (max)
Group	Patient	4.60	3.55	3.70	0.65	22.02
	Control	1.03	0.67	0.88	0.20	2.50

ficant difference in LYVE-1 protein levels between lung cancer patients and healthy controls at a significance level of p=0.05.

RESULTS

Serum protein LYVE1 levels were found to be higher in patients with lung cancer compared with the healthy control group, and a statistically significant difference was determined (p=0.001). The gene of *LYVE-1* mean (x), standard deviation (sd) and median (m) values are 4.60±3.55; 3.70 ng/ml and in healthy controls it was 1.03±0.67; 0.88 ng/ml. *LYVE-1* gene expression levels showed statistical significance when compared with the healthy control group (p=0.001) (Table 2 and 3).

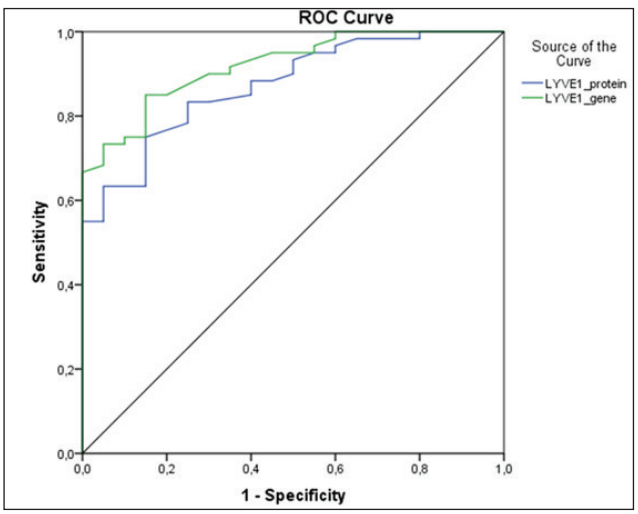
ROC analysis was performed for each test. Consequently, ROC analysis, AUC values for LYVE-1 protein and gene expression levels in NSCLC cancer patients were calculated, 0.892 and 0.920 respectively (Figure 1). Based on the AUC, the diagnostic value of *LYVE-1* gene expression was higher than the LYVE-1 protein level.

Boxplots depicting the variations in serum markers between patients with lung cancer and healthy controls are presented. Serum LYVE-1 protein is shown by boxplots with p<0.05 between NSCLC patients and healthy controls. The centre line in boxplots indicates the median for each data set (Figure 2 and 3).

According to Spearman's rank correlation test, a good correlation was found between the LYVE-1 protein and *the LYVE-1* gene. (Spermans Spearman's rho 0.690 p-value <0.001).

Table 4 displays the statistical significance between the serum LYVE-1 protein and gene expression values and various clinico-

Area Under the Curve



Test Result Variable(s)	Area
LYVE1_protein	.873
LYVE1_gene expression	.920

Figure 1: Receiver operating characteristic (ROC) curves for each test. The area under the curve for the LYVE-1 protein and LYVE-1 gene were 87.3% and 92.0%, respectively.

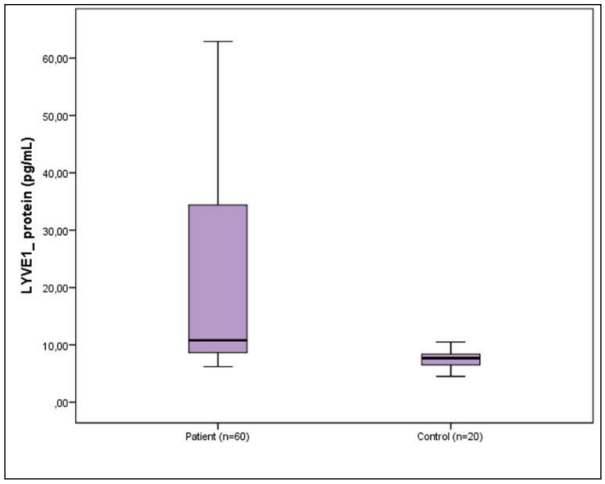


Figure 2: Serum LYVE-1 protein levels (ng/ml) in lung cancer patients and controls (p=0.001)

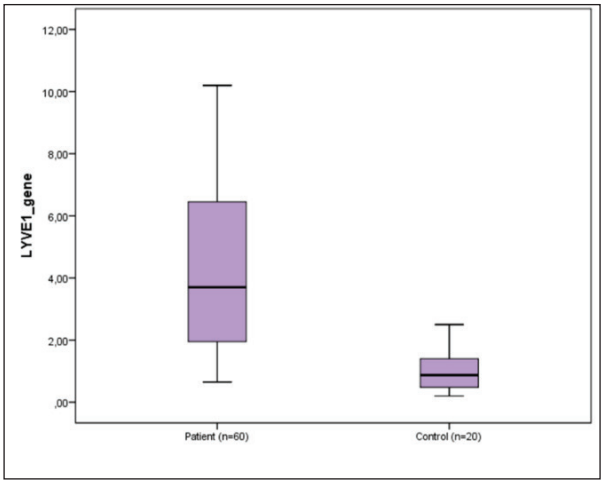


Figure 3: Serum LYVE-1 gene levels (ng/ml) in lung cancer patients and controls (p=0.001)

Table 4: Results of comparisons between serum LYVE-1 protein LYVE-1 gene expression and various demographic and disease

Characterictics		Number of patients	P value	P value
Age	<60	30	0.450	0.510
	>60	30		
Smoking	Yes	35	0.310	0.420
	No	25		
Gender	Male	48	0.550	0.510
	Female	12		
Stage	III	28	0.802	0.251
	IV	32		
Metastasis	Yes	32	0.782	0.232
	No	28		
Histological	NSCLC	50	0.732	0.689
	SCLC	10		
Type	Adeno	30	0.796	0.136
	Squamoz	30		

SCLC: Small cell lung cancer
P value: Statistical significance (Mann-Whitney U testi)
NSCLC: Non-small-cell lung cancer

pathological variables. As for both serum LYVE-1 protein and gene expression of these variables age, stage, gender, metastasis, histological type showed is not a statistically significant with the level of the biomarker. Our results indicate a concordance between LYVE-1 protein levels and mRNA expression in the serum of both control and lung cancer patients. Notably, both protein and mRNA expression levels were found to be significantly higher in the control group than in the lung cancer patient group.

DISCUSSION

Numerous biomarkers have been used clinically, with many protein-based assays readily available. Advances in the application of specific antibodies have significantly enhanced clinical diagnosis using biomarkers. While proteomic studies conducted worldwide have employed comprehensive protein-based approaches to identify improved biomarkers, the detection rates of traditional protein biomarkers have reached their limits.

We proposed circulating mRNA as a novel biomarker. mRNA is a valuable molecule. This method is capable of detecting genes with low protein expression levels as well as non-coding RNA precursors and gene products with limited cellular secretion. Although circulating mRNA is generally considered unstable in RNase-rich blood, our findings confirm that mRNA levels remain stable for at least 24 h after blood sample collection.

In contrast to DNA, RNA molecules possess the unique ability to indicate dynamic, daily physiological and pathological processes occurring within the human body, providing opportunities for early disease detection (15). In this study, we demonstrated the feasibility of detecting and quantifying mRNAs circulating in the human bloodstream.

Lung cancer has the highest mortality rate worldwide. In spite of the fact that the adequacy of diagnosis and treatment in lung cancer has improved recently. As of late, there is developing evidence that identifying biomarkers can progress lung cancer diagnosis, guess and treatment. Thus, the search for novel biomarkers can advance the conclusions and prediction of lung cancer. In our research, circulating mRNA has proven to be an exceptionally sensitive molecule for detecting diseases and systemic inflammatory conditions. Nevertheless, this approach has certain limitations. It may pose challenges in cases involving organs with high blood flow, such as the liver, which could be affected by disease progression. In addition, the target disease may remain latent, as observed in patients with progressive liver conditions. Considering these challenges, it is anticipated that advancements in circulating RNA research in the coming years will pave the way for novel diagnostic and monitoring approaches, enhancing our ability to detect and manage diseases effectively (16).

Through cell culture studies, *LYVE-1* genes will be identified. This study can determine when they begin to express themselves in vascular structures. Therefore, further research can be designed. Research can be planned according to different cultures. - Migration to show the link between CD44 and the immune system Establishment of LYVE-1 as a specific biomarker. for lymphatic vasculature presents a potential minimally invasive approach (17). Quantifying LYVE-1 levels can yield valuable information for the diagnosis of lung cancer. Various methods have been employed to detect LYVE-1, an integral membrane glycoprotein (18, 19). In this study, real-time PCR was used as the molecular method for LYVE-1 assessment. Over the past decade, there has been increasing attention towards the development of rapid diagnostic techniques. However, the growing sensitivity of detection methods introduces a challenge in differentiating between insignificant alterations and lesions that may progress to malignant cancer. In the past decade, there has been a growing focus on the development of rapid diagnostic techniques. However, the enhanced sensitivity of the detection methods presents a challenge in distinguishing between inconsequential alterations and lesions that can progress to malignant cancer.

LYVE-1 is specifically found in the lymphatic capillary endothelium and becomes active in the endothelium of the spleen, liver sinusoidal endothelial cells (LSEC), and macrophages (19, 20). During cancer progression, the presence of LYVE-1 increases within the endothelial cells of the lymphatic system. A dense concentration of LYVE-1-expressing lymphatic vessels is associated with an elevated occurrence of regional lymph node metastases (21).

LYVE-1 is emerging as a valuable diagnostic and prognostic biomarker in a range of cancers (22, 23). LYVE-1 plays a crucial role in processes such as hyaluronic acid homeostasis and the regulation of migration to the lymph nodes. The lymphatic system serves as a key pathway for early-stage cancer metastasis. LYVE-1 has been extensively used to identify tumour-associated lymphatic vessels in various cancer types. The intensity of LYVE-1 expression, as determined through immunohistochemistry, is an important tool for assessing lymphatic system infiltration and lymphangiogenesis in cancers (24). Based on the existing literature, our study revealed that both the protein and mRNA levels of LYVE-1 were significantly elevated in lung cancer patients compared to healthy controls.

Lymphangiogenesis is considered a key indicator for evaluating metastasis and predicting the prognosis of patients with gastric cancer. This study assessed the potential of LVD-tagged LYVE-1 to enhance lymphangiogenesis. Immunohistochemical analysis revealed that LVD in the plant-based medicinal food treatment with the dense-dose group showed a significantly lower incidence of recurrent tumours. These findings suggest hat PBMF exerts an inhibitory effect on lymph node formation (25).

In a cell culture study by Prevo et al., LYVE-1 was capable of binding and internalising hyaluronan *in vitro*, providing valuable insights into the potential role of LYVE-1. Although it is not yet confirmed whether LYVE-1 circulates in vivo, substantial ectodomain fragments of LYVE-1 have been identified (26). In our research, we used readily accessible serum samples from patients with lung cancer. Our findings revealed that serum LYVE-1 levels exhibited an inverse relationship with the size of the primary tumour and served as a significant predictor of both lymph node involvement and distant metastases. Moreover, lung cancer patients with lower serum LYVE-1 levels demonstrated poorer prognoses compared with those with higher levels (27).

Additionally, Two et al. proposed that LYVE-1 enhanced the adhesion of hyaluronan-high-expressing HS-578T cells to LYVE-1-transfected COS-7 cells (28). Similarly, Ito et al. observed elevated levels of hyaluronan on the surfaces of highly metastatic murine breast carcinoma cells and their surrounding stroma, with lymph node-infiltrating breast cancer cells expressing high levels of LYVE-1 via hyaluronic acid in vivo (29).

One of the advantages of our study is that the determination of LYVE-1, which can be a biomarker among those with advanced stage lung cancer at both the protein level and gene expression from an easily obtainable serum sample, will contribute to the literature.

LYVE1 expression was found to be elevated in tissue from patients with lung cancer, with significantly higher protein levels of the lymphoid-specific marker LYVE-1 observed in these samples compared with normal tissue. Immunohistochemical analysis further confirmed that *the LYVE-1* gene was expressed at higher levels in lung cancer tissues than in normal tissues (30). Similarly, our study was designed using serum instead of tissue samples, and LYVE-1 mRNA levels were found to be significantly higher in lung cancer patients compared with the control.

Our study has several limitations. First, we did not conduct LYVE-1 immunostaining on tumour. Secondly, cytokine levels were not assessed in patient blood samples, even though these levels are likely to exhibit changes relative to serum LYVE-1 levels in lung cancer patients. Third, although we evaluated serum LYVE-1 levels in lung cancer patients before and following treatment, further research is required to evaluate the temporal effects of therapy on these levels.

This study showed that both serum LYVE-1 protein and mRNA levels were significantly higher in patients with lung cancer. The current research demonstrated that increased levels of serum LYVE-1 protein, as well as mRNA, were markedly elevated in individuals diagnosed with lung cancer. LYVE-1 protein levels were not correlated with the stage of NSCLC, tumour size, or the presence of metastasis. These findings suggest that both the LYVE-1 protein and gene may have diagnostic value for advanced lung cancer. Despite the fact that the serum values of both LYVE-1 protein and gene are elevated in lung cancer patients, the *LYVE-1* gene is of more diagnostic importance than the results of the ROC analysis according to sensitivity and specificity.

To the best of our knowledge, *LYVE-1* serum mRNA expression in patients with advanced lung cancer has not been previously studied. We found that LYVE-1 was under-expressed in the blood of patients with NSCLC. In contrast to the literature that has reported its overexpression in various solid tumours. The present study contributes to the literature in that, to the best of our knowledge, this study is one of the studies examining the serum LYVE-1 protein and gene and their combined levels in lung cancer patients. Further trials with larger patient populations are necessary to determine the clinical importance of these biomarkers in patients with lung cancer. This study investigated the mRNA and protein levels of *LYVE-1* mRNA in patients with lung cancer.

CONCLUSION

The management of lung cancer has increasingly shifted towards a biomarker-driven approach. With the rapid development of effective targeted therapies, efforts have been made by organisations to establish best practices regarding the necessary tests and their appropriate target populations. Targeted testing enhances the coverage of relevant genomic regions, facilitates the identification of significant alterations, and ensures that crucial molecular data are available in a timely manner to guide therapeutic decision-making.

Serum-based assays provide several advantages compared with tissue-based tests.including being non-invasive, rapid, and easily reproducible over time. However, they may exhibit lower sensitivity compared with tissue-based tests and, therefore, cannot serve as a standalone diagnostic tool for patients with NSCLC. A range of tissue- and blood-based assays are available for biomarker analysis, each possessing distinct advantages and limitations that clinicians must consider when selecting the appropriate assay. Genomic abnormalities related to tumours, identified through biopsy or plasma analysis, provide comparable benefits. Studies based on non-invasive methods using patient blood are highly valuable for monitoring treatment response and detecting the emergence of acquired resistance before radiographic or clinical progression becomes evident.

The recognition of LYVE-1 as an accurate biomarker for lymphatic vasculature presents a potential non-invasive strategy for managing patients with lung cancer. The identification and measurement of LYVE-1 can yield crucial information for lung cancer diagnosis in clinical practice. Several methods exist to identify the LYVE-1 molecule, including immunohistochemical techniques. In the past decade, significant attention has been devoted to developing rapid techniques for its detection. However, enhancing the sensitivity of these detection methods remains a challenge, particularly in distinguishing between substantial changes and lesions that could progress to malignant cancer.

Ethics Committee Approval: This study was approved by İstanbul University Ethics Committee (Date: 27.08.2014, No: 1311).

Informed Consent: Written informed consent was obtained from all the participants of the study.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- M.S., D.D., U.G., F.T.; Data Acquisition- M.S., F.T., C.T.Y.; Data Analysis/Interpretation- M.S., C.T.Y., H.O.S., E.Ö.; Drafting Manuscript- M.S., C.T.Y., H.O.S., E.Ö.; Critical Revision of Manuscript- M.S., D.D., U.G., F.T.; Final Approval and Accountability- M.S., C.T.Y., H.O.S., E.Ö., D.D.; Material and Technical Support- M.S., E.Ö., C.T.Y.; Supervision- M.S., F.T., D.D., U.G., H.O.S.

Conflict of Interest: The authors declare that there is no conflict of interest.

Financial Disclosure: The present work was supported by the Research Fund of Istanbul University. (49520)

REFERENCES

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin 2013;63:11-30.
2. Yilmaz S, Dogan Sarikaya M, Yasar E, Sen Burcu B, Canoz O, Onal O. determination of metastatic capacity in primary lung cancer cells: reflection of patient profile in the clinic using in vitro methods. Journal of Advanced Research in Health Sciences 2023;6(3):302-11.
3. Witte MH, Witte CL. Lymphatics and blood vessels, lymphangiogenesis and hemangiogenesis: from cell biology to clinical medicine. Lymphology 1987;20(4):257-66.
4. Gerli R, Solito R, Weber E, Agliano M. Specific adhesion molecules bind anchoring filaments and endothelial cells in human skin initial lymphatics. Lymphology 2000;33(4):148-57.
5. Ryan TJ. Structure and function of lymphatics J Invest Dermatol (Suppl) 1989;93:18–24.
6. Skobe M, Detmar M. Structure, function, and molecular control of the skin lymphatic system. J Invest Dermatol Symp Proc 2000;5:14–9.
7. Von Andrian UH, Mempel TR. Homing and cellular traffic in lymph nodes. Nat Rev Immunol 2003;3(11):867-78.
8. Stacker SA, Achen MG, Jussila L, Baldwin ME, Alitalo K. Lymphangiogenesis and cancer metastasis. Nat Rev Cancer 2002;2(8):573-83.
9. Oliver G, Sosa-Pineda B, Geisendorf S, Spana EP, Doe CQ, Gruss P. Prox1, a prospero-related homeobox gene expressed during mouse development. Mech Dev 1993.44(1):3-16.
10. Nakamura K, Rockson SG. Biomarkers of lymphatic function and disease. Mol Diag Ther 2007;11(4):227-38.
11. Wigle, JT, Oliver G. Prox1 function is required for the development of the murine lymphatic system. Cell 1999;98(6):769-78.
12. Banerji S, Ni J, Wang SX, Su J, Tammi R, Jones M, Jackson DG. LYVE-1, a new homologue of the CD44 glycoprotein, is a lymph-specific receptor for hyaluronan. J Cell Biol 1999;144:789–801.
13. Prevo R, Banerji S, Ferguson D, Jackson DG. Mouse LYVE-1 is an endocytic receptor for hyaluronanin lymphatic endothelium. J Biol Chem 2001;276:19420–30.
14. Jackson DG. The lymphatics revisited: new perspectives from the hyaluronan receptor LYVE-1. Trends Cardiovasc Med 2003;13:1–7.

15. Dhama K, Latheef SK, Dadar M, Samad H, Munjal A, Khandia R, et al. Biomarkers in stress related diseases/disorders: diagnostic, prognostic, and therapeutic values. Front Mol Biosci 2019;18:691.
16. Wishart DS, Bartok B, Oler E, Liang KY, Budinski Z, Berjanskii M, et al. An online database of molecular biomarkers. Nucleic Acids Res 2021;49:D1259–67.
17. Jauch AS, Wohlfeil SA, Weller C, Dietsch, V, Stojanovic A, Kittel M, et al. Lyve-1 deficiency enhances the hepatic immune microenvironment entailing altered susceptibility to melanoma liver metastasis Cancer Cell Int 2022;22(1):398.
18. Lim HY, Lim SY, Tan CK, Thiam CH, Goh CC, Carbajo D, et al. Hyaluronan receptor LYVE-1-expressing macrophages maintain arterial tone through hyaluronan-mediated regulation of smooth muscle cell collagen. Immunity 2018;49(6):1191.
19. Fujimoto A, Ishikawa Y, Akishima-Fukasawa Y, Ito K, Akasaka Y, Maehara S, et al. Significance of lymphatic invasion on regional lymph node metastasis in early gastric cancer using LYVE-1 immunohistochemical analysis. J Clin Pathol 2007;127:82–8.
20. Wrobel T, Dziegiel P, Mazur G, Zabel M, Kuliczkowski K, Szuba A. LYVE-1 expression on high endothelial venules (HEVs) of lymph nodes. Lymphology 2005;38:107–10.
21. Carreira CM, Nasser SM, Tomaso E, Padera TP, Boucher Y, Tomarev SI, et al. LYVE-1 is not restricted to the lymph vessels: expression in normal liver blood sinusoids and down-regulation in human liver cancer and cirrhosis. Cancer Res 2001;61:8079–84.
22. Ozmen F, Ozmen M, Ozdemir EM, Moran M, Seçkin S, Guc D, et al. Relationship between LYVE-1, VEGFR-3 and CD44 gene expressions and lymphatic metastasis in gastric cancer. World J Gastroenterol 2011;17:3220–8.
23. Giatromanolaki A, Sivridis E, Koukourakis MI. Angiogenesis in colorectal cancer: prognostic and therapeutic implications. Am J Clin Oncol 2005;58:202–6.
24. Nunomiya K, Shibata Y, Abe S, Inoue S, Igarashi A, Yamauchi K, et al. Relationship between serum level of lymphatic vessel endothelial hyaluronan receptor-1 and prognosis in patients with lung cancer J. Cancer 2014;5(3):242-7.
25. Yoo YA, Kang MH, Lee HJ, Kim B, Park JK, Kim HK, et al. Sonic hedgehog pathway promotes metastasis and lymphangiogenesis via activation of Akt, EMT, and MMP-9 pathway in gastric cancer. Cancer Res 2011;71(22):7061–70.
26. Prevo R, Banerji S, Ferguson DJ, Clasper DS, Jackson DK. Mouse LYVE-1 is an endocytic receptor for hyaluronan in lymphatic endothelium. JBC Papers in Press 2001;276(22):19420-30.
27. Nunomiya K, Shibata, Abe S, Inoue S, Igarashi A, Yamauchi K, et al. Relationship between serum level of lymphatic vessel endothelial hyaluronan receptor-1 and prognosis in patients with lung. Cancer J Cancer 2014;5(3):242-7.
28. Du Y, Liu H, He Y, Liu Y, Yang C, Zhou M, et al. The interaction between LYVE-1 with hyaluronan on the cell surface may play a role in the diversity of adhesion to cancer cells. PLoS One 2013;8(5).
29. Shibata MA, Hamaoka H, Kanayama T, Maemura K, Ito Y, Linuma M, et al. Synthetic a-mangostin dilaurate strongly suppresses widespectrum organ metastasis in a mouse model of mammary cancer. Cancer Sci 2018;109(5):1660–71.
30. PenG LI, Cong Z, Yong Qiang, Lei Xiong, Tang L, Zhang Y, et al. Significance of CCBE1 expression in lung cancer. Mol Med Rep 2018;17(2):2107-12.