JOSIP JURAJ STROSSMAYER UNIVERSITY OF OSIJEK
FACULTY OF MEDICINE OSIJEK

Gramos Begolli

Serum Tumor Markers in the Early Stage of Lung Cancer and in the Non-malignant Pulmonary Inflammations

Doctoral Dissertation

Osijek, 2024.

JOSIP JURAJ STROSSMAYER UNIVERSITY OF OSIJEK
FACULTY OF MEDICINE OSIJEK

Gramos Begolli

Serum Tumor Markers in the Early Stage of Lung Cancer and in the Non-malignant Pulmonary Inflammations

Doctoral Dissertation

Osijek, 2024.

Mentor: Assoc. Prof. Željko Debeljak, PhD

Co-Mentor: Assist. Prof. Ilijan Tomaš, PhD

The dissertation contains 83 pages.

Preface:

I would like to extend my deepest gratitude to all those who have supported me throughout this journey.

First and foremost, I am sincerely grateful to my mentor Assoc. Prof. Željko Debeljak, whose unwavering guidance, invaluable insights, and encouragement have been instrumental in shaping this study. His mentorship provided not only academic direction but also personal inspiration.

I would also like to express my sincere appreciation to the co mentor of this work Ass. Prof. Ilijan Tomaš, whose constructive feedback and thoughtful suggestions greatly enhanced the quality and rigor of this study.

A special thank is extended to all collogues from Clinical Institute of Laboratory Diagnostics in University Hospital Center Osijek, for their collaboration and providing thenecessary resources, as well as a conducive environment for this study. Clinical HospitalCenter Zagreb for their contribution to data collection and access to relevant patient information was vital to the success of this research.

To my parents Besnik and Luljeta, whose support and belief in me have been a constantsource of strength throughout my academic career, I owe much of my success.

Finally, to my wife Donika, and my children, Diesa and Diell, I am profoundly grateful for their patience, understanding, and unwavering love. Their support throughout this journeyhas been immeasurable, and without them, this achievement would not have been possible.

Contents: **Page number**

Abbreviations

ADC	Adenocarcinoma
AI	Artificial Intelligence
ALK	Anaplastic Lymphoma Kinase
ARDS	Acute Respiratory Distress Syndrome
AUC	Area-Under-Curve
BALF	Bronchoalveolar Lavage Fluid
CEA	Carcinoembryonic Antigen
CHST	Carbohydrate Sulfotransferases
COPD	Chronic Obstructive Pulmonary Disease
COPD_E	Chronic Obstructive Pulmonary Disease Exacerbation
COPD_R	Chronic Obstructive Pulmonary Disease Remission
CSPG	Chondroitin Sulfate Proteoglycan
CT	Computed Tomography
cfDNA	Cell-free DNA
ctDNA	Circulating Tumor DNA
CYFRA21-1	Cytokeratin 19 Fragment
ECLIA	Electrochemiluminescence Immunoassay
EGFR	Epidermal Growth Factor Receptor
ELISA	Enzyme Linked Immunoassay

GAG	Glycosaminoglycan
GOLD	Global Initiative for Chronic Obstructive Lung Disease
IL-6	Interleukin 6
IL-8	Interleukin 8
KRAS	Kirsten Rat Sarcoma Virus
LCLC	Large Cell Lung Carcinoma
LDCT	Low-Dose Computed Tomography
MRI	Magnetic Resonance Imaging
MWU	Mann-Whitney U
NETs	Neuroendocrine Tumors
NSCLC	Non-Small Cell Lung Cancer
NSE	Neuron-Specific Enolase
O.D.	Optical Density
PAPS	3′Phosphoadenosine-5′ Phosphosulfate
PET	Positron Emission Tomography
proGRP	Progastrin-Releasing Peptide
ROC	Receiver Operating Characteristic
ROS	Reactive Oxygen Species
SCLC	Small Cell Lung Cancer
SULTs	Sulfotransferases
SQCC-SCC	Squamous Cell Carcinoma

TNF-α	Tumor Necrosis Factor Alpha
TNM	Tumor, Node, Metastasis
TILs	Tumor-Infiltrating Lymphocytes
WHO	World Health Organization

List of Tables

TABLES:

List of figures:

1. BACKGROUND

1.1. Association between Inflammation, COPD, and Lung Cancer

Lung cancer and chronic obstructive pulmonary disease (COPD) are among the most significant and prevalent health challenges worldwide, with both diseases contributing to substantial morbidity and mortality (1). These conditions share common underlying mechanisms of inflammation, including the activation of inflammatory cells, which play a pivotal role in their pathophysiology (2) and often present with initial symptoms, complicating the diagnostic process. Both conditions may share clinical manifestations, including persistent cough, shortness of breath, chest pain, and fatigue. This symptom overlap can lead to challenges in early differentiation, as these symptoms are commonly associated with various respiratory disorders. Additionally, inflammatory conditions like pneumonia and COPD may frequently coexist with lung cancer, further complicating the diagnostic landscape. The presence of such comorbidities can lead to misinterpretation of symptoms and challenge clinicians in distinguishing between malignant and benign pulmonary diseases. In fact, it is not uncommon for these inflammatory conditions to be initially misdiagnosed as lung cancer, particularly in patients with risk factors for malignancy (3). The lungs, constantly exposed to various pathogenic agents such as pollutants, infections, and irritants, initiate a rapid and robust immune response aimed at neutralizing these threats (1). In the process of inflammation, cytokines and other inflammatory mediators are released into the tissues, leading to alterations in cellular functions and fostering an environment that amplifies the inflammatory cascade (1). This intricate coordination among cellular and molecular components of the immune system serves not only to manage immediate threats

but also to propagate the inflammation. Pulmonary inflammation can manifest acutely, as seen in conditions such as pneumonia and acute respiratory distress syndrome (ARDS), or can become chronic, as observed in asthma and COPD (4,5). Chronic inflammation in COPD, in particular, is a critical risk factor for the development of lung cancer, as it induces oxidative stress, promotes DNA mutations, elevates pro-inflammatory cytokine levels, impairs DNA repair mechanisms, and fosters abnormal cell proliferation, thereby increasing the likelihood of malignant transformation (6). The overlapping inflammatory pathways and molecular alterations in both COPD and lung cancer highlight the need for comprehensive diagnostic strategies and therapeutic interventions that can address both conditions concurrently.

1.2. Common Pathophysiology of Lung Cancer and Lung Inflammations

Chronic inflammation plays a central role in the pathophysiology of both lung cancer and COPD. The primary triggers of inflammation in the lungs are environmental factors such as cigarette smoke, air pollution, and microbial pathogens, which lead to the recruitment of various inflammatory cells into lung tissues (1, 7). These cells, including macrophages, neutrophils, and lymphocytes, release a range of pro-inflammatory cytokines such as Tumor Necrosis Factor alpha (TNF-alpha), Interleukin-6 (IL-6), and Interleukin-8 (IL-8) (8). These cytokines not only attract additional inflammatory cells to the site of injury but also activate intracellular signaling pathways that are involved in cell proliferation, survival, and angiogenesis. As the inflammatory response progresses, it generates BA and increases oxidative stress within the lung tissue. This oxidative environment leads to DNA damage, mutations, and the eventual onset of cancer (9, 10).

Moreover, inflammation impairs the DNA repair mechanisms, further exacerbating the accumulation of mutations that can contribute to carcinogenesis. The persistent inflammatory microenvironment also facilitates the release of growth factors, cytokines, and chemokines, which are crucial for tumor growth, angiogenesis, and the invasive and metastatic potential of cancer cells. Thus, the pathophysiological interactions between COPD, inflammation, and lung cancer progression underscore the pivotal role of inflammation in the development and advancement of lung cancer. Given this, targeting inflammatory pathways presents a promising approach for both the prevention and treatment of lung cancer. Reducing chronic inflammation, or modulating the immune response to better regulate the inflammatory process, may significantly decrease the risk of lung cancer onset and progression, making inflammation a crucial therapeutic target in combating lung cancer.

1.3. Early Detection of Lung Cancer

Interactions between COPD, chronic inflammation, and lung cancer highlights critical mechanisms that are essential for understanding the progression, prevention, and management of these interconnected diseases (11). Chronic inflammation associated with COPD not only exacerbates pulmonary dysfunction but also creates a microenvironment conducive to carcinogenesis, involving oxidative stress, DNA damage, impaired repair mechanisms, and the release of pro-inflammatory cytokines. This relationship underscores the importance of implementing effective strategies to manage COPD and mitigate chronic inflammation, which may significantly reduce the risk of lung cancer development and associated mortality (12).

Proactive measures aimed at controlling inflammation and addressing risk factors, such as smoking cessation, air quality improvement, and timely treatment of infections, could play a pivotal role in breaking this pathogenic link. Additionally, advancing diagnostic techniques for the early detection of lung cancer is imperative (13). Early-stage lung cancer diagnosis enables accurate staging, facilitates the selection of appropriate and effective treatment strategies, and significantly improves patient outcomes by preventing the disease from progressing to a life-threatening stage (14, 15).

Lung cancer remains a leading cause of cancer-related mortality worldwide, posing a significant challenge to global health. Early diagnosis is particularly critical, as detecting the disease in its initial stages can greatly improve prognosis, increase treatment efficacy, and ultimately enhance life expectancy (16). Despite advancements in medical technologies and screening programs, the early identification of lung cancer remains suboptimal, contributing to poor survival rates, particularly in advanced stages.

According to the latest World Health Organization (WHO) classification, lung cancer is categorized into two primary histological types: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC, which accounts for approximately 85% of all cases, includes subtypes such as adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, each with distinct biological behaviors and clinical characteristics (17). In contrast, SCLC represents about 15% of lung cancer cases and is characterized by its rapid growth, early metastasis, and strong association with smoking. This histological distinction is critical not only for diagnosis but also for selecting appropriate treatment strategies, as therapeutic approaches for NSCLC and SCLC differ significantly (Figure 1). Improved understanding of the histopathological and molecular characteristics of lung cancer is

essential for advancing early diagnostic methods and tailoring personalized treatment approaches.

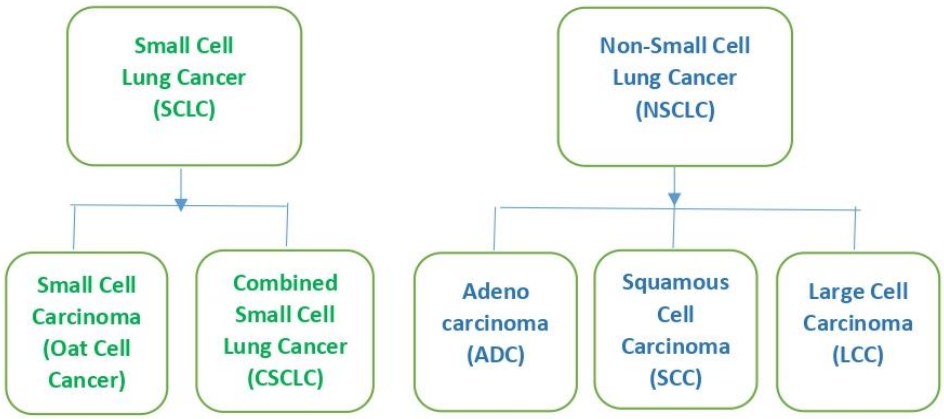

Figure 1. Types of Lung Cancer. Schematic presentation of the two main types of lung cancer: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). SCLC is divided into: Small Cell Carcinoma (Oat Cell Cancer) and Combined Small Cell Lung Cancer (CSCLC) while NSCLC is divided into: Adeno carcinoma (ADC) Squamous Cell Carcinoma (SCC) Large Cell Carcinoma (LCC). (Source: created by the author of the dissertation).

NSCLC is further categorized into several subtypes, each with distinct characteristics, clinical presentations, and associations with risk factors. Adenocarcinoma (ADC) is the most common subtype, accounting for the majority of NSCLC cases. ADC typically arises in the peripheral regions of the lungs and is more frequently observed in individuals who rarely smoked or those who have quit smoking for a significant period (18). It is characterized by glandular differentiation and the production of mucin, which can often be detected through histopathological examination. The relatively high prevalence of ADC among non-smokers underscores the need to identify additional environmental and genetic risk factors beyond smoking.

Another major subtype of NSCLC is squamous cell carcinoma (SQCC), which is strongly associated with long-term tobacco use. SQCC generally develops in the central regions of the lungs, often originating in the bronchial tubes, and is characterized histologically by keratinization and the formation of intercellular bridges (19, 20). Its strong correlation with smoking highlights the importance of continued public health efforts focused on smoking cessation to reduce lung cancer incidence. In contrast, large cell carcinoma (LCLC) and SCLC are less common but notable for its aggressive clinical course, rapid tumor growth, and higher likelihood of metastasis.

Lung cancer can also be staged depending on the extent of tumor invasion into lung structures and adjacent or distant organs and lymph nodes. The TNM (Tumor, Node,and Metastasis) staging system is widely used to classify the stage of lung cancer, for treatment plans and outcome assessments (12) (Table 1). The TNM staging uses the extent of the tumor (T), involvement of regional lymph nodes (N), and metastasis to distalorgans (M). It is important to understand which stage of lung cancer the patient is in whenchoosing the right treatment plan and to estimate the further prognosis (21, 22). Proper staging is essential in guiding treatment strategies that suit a particular patient, in an attempt to increase the survival and quality of life (21, 22).

COPD is linked to persistent inflammation, which can cause alterations in lung tissue such as fibrosis or the development of benign nodules. These alterations can imitate or mask the existence of a tumor, making the diagnostic procedure more complex(23). In COPD and in acute pulmonary inflammation, there is an overproduction of mucusand blockage of the airways, which can make it difficult to detect tumors on imaging investigations, especially CT scans (24). This can make small tumors challenging to detect or result in misdiagnosis.

Hyperplasia of epithelial cells, which may exhibit similarities to neoplastic development in biopsy samples, can complicate the diagnostic process as it requires careful evaluation to distinguish between benign proliferative changes and early signs of malignant transformation. The presence of inflammatory cellsand necrosis in acute inflammation might also make the histological interpretation more complex (24). Both COPD and lung tumors can have similar radiographic characteristics,including the presence of nodules, mass-like opacities, or consolidation on imaging (25). The similarity in imaging results introduces difficulties in differentiation between malignantand non-malignant lesions (25).

Table 1. Lung Cancer TNM Staging (Source: created by the author of the dissertation).

	T1	**T2**	**T3**	**T4**
Tumor size	< 3 cm	3 -7 cm Atelectasis (part of lung) Invasion: Visceral pleura, main bronchus ≥ 2 cm from carina	> 7 cm Atelectasis (whole lung) Invasion: Phrenic nerve, diaphragm, chest wall, mediastinal pleura, main bronchus < 2 cm from carina, parietal pericard	Invasion mediastinal organs/vertebral bodies/carina/tumor nodules in different ipsilateral lobe
	N0	**N1**	**N2**	**N3**
Lymph node	No lymph nodes involvement	Ipsilateral bronchopulmonary/hilar	Ipsilateral mediastinal/subcranial	Contralateral hilar/ contralateral mediastinal/ supraclavicular
	M0	**M1**		
Metastasis	No metastasis	Bilateral lesions Distant metastasis Malignant pleural effusion		

1.4. Diagnostic and prognostic methods used in lung cancer patients

The current approach to lung cancer diagnostics is comprised by utilization of a wide variety of imaging tools including chest radiography, computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET) scans in conjunction with the pathological examination of biopsy samples, but these techniques frequently cannot detect early lung cancer (26). The low-dose computed tomography (LDCT) scan is currently the preferred method for screening since it is a simple and quick examination of the chest that does not include the use of contrast substances (27). Artificial intelligence (AI) methods like machine learning and deep learning have been recently introduced in detection and characterization of lung nodules, to improve lung cancer screening and diagnosis by correct classification using low-dose CT scans, PET-CT imaging, and chest radiographs.

New laboratory approaches for lung cancer diagnostics have been developed to improve the detection rate. Circulating tumor DNA (ctDNA) is widely used in the diagnosis of lung cancer. ctDNA is a sub-type of cell-free DNA (cfDNA) that circulates in the bloodstream after being shed off from tumor cells (28). Liquid biopsy through cfDNA analysis allows testing for tumor-specific genetic changes and mutations. Gobbini et al. have indicated that ctDNA can detect genetic alterations such as the epidermal growth factor receptor (EGFR), Kirsten Rat Sarcoma virus (KRAS) and *anaplastic lymphoma kinase* (*ALK*) genes with acceptable sensitivity and selectivity, enabling the customization of the patient's treatment (29). Another new technology, namely small interfering RNA (siRNA) technology, effectively targets the genes that aid tumor formation and malignancy (30). siRNA-based diagnostics may help in development of molecular profiles of lung

cancer when oncogenes or tumor suppressor genes are targeted.

DNA methylation profiling is yet another new laboratory technique in lung cancer diagnostics (31). Cancer development is characterized by unique DNA methylation profiles that may be used as biomarkers of diagnosis and survival rates. Methylation- specific PCR and bisulfite sequencing are the most common methods to study such changes (32). Certain methylation markers, including *RASSF1A* and *CDKN2A* genes, arespecific and sensitive for differentiating malignant from benign lung nodules and for the prognosis of patients (33). ctDNA analysis, siRNA profiling, and DNA methylation analysis are promising tools for practicing precision medicine in lung cancer. The approaches facilitate assessing processes during disease initiation, simultaneous monitoring of disease processes, and developing individually selected therapeutic interventions, positively influencing clinical results and the diagnostics of lung cancer.

Furthermore, comprehensive global lipidomic analyses have revealed distinct plasma lipid profiles that are strongly associated with early-stage lung cancer, highlighting their potential as novel biomarkers for early detection (34). A study published by Yu et all, demonstrated that specific lipid markers exhibit significant differences between patients with early-stage NSCLC and healthy individuals, suggesting their diagnostic utility in distinguishing malignant cases at an early stage. By employing advanced mass spectrometry techniques, there were identified key lipid alterations that could serve as reliable indicators of early lung cancer development. These findings pave the way for the establishment of a minimally invasive, rapid, and accurate blood-based diagnostic test, which could facilitate early intervention and improve patient outcomes. The ability to detect lung cancer in its initial stages through plasma lipid profiling represents a significant advancement in the field of cancer diagnostics

(34), underscoring the crucial role of lipid metabolism in tumorigenesis and the growing importance of lipidomics in precision medicine.

AI models can also improve early detection by analysis of serum biomarkers and tumor markers (35), according to Kim et al., machine learning-based predictive modeling utilizing bronchoalveolar lavage fluid (BALF) microbiome characteristics demonstrated a significant ability to differentiate lung cancer from benign pulmonary diseases (36). This advanced analytical approach effectively identified distinct microbial signatures associated with malignant and non-malignant conditions, highlighting the potential of BALF microbiome profiling as a valuable tool for lung cancer detection. The findings suggest that alterations in microbial composition within the lung environment may serve as crucial indicators of tumor presence, potentially reflecting the interplay between microbial dysbiosis and oncogenic processes. Given the growing recognition of the microbiome’s role in cancer pathogenesis, these results underscore the potential of BALF microbiome analysis as a non-invasive and highly specific biomarker for lung cancer diagnosis (35, 36).

1.5. Serum Protein Biomarkers Routinely Used in Lung Cancer Patient Work-Up

Pulmonary malignancies are a diverse group of cancers that vary greatly in their nature, etiology, and molecular characteristics, making their diagnosis particularly challenging. This complexity necessitates the use of multiple diagnostic biomarkers, as emphasized by Vansteenkiste et al. (37, 38), to ensure accurate and timely detection. One of the primary difficulties in diagnosing pulmonary malignancies is that the molecular descriptors of these cancers often overlap with other respiratory conditions, such as COPD and pulmonary infections (39). This overlap can complicate the clinical decision-making process, as distinguishing between benign and malignant lung conditions becomes more difficult based solely on traditional diagnostic methods. Despite these challenges, serum protein tumor markers have gained traction in clinical practice as valuable tools, as they can provide critical information for the early detection of lung cancer and help with the risk stratification of patients (40, 41).

Early detection of lung cancer is crucial because it significantly impacts treatment outcomes. Biomarkers, particularly protein tumor markers, can serve as a non-invasive and relatively safe method for identifying the presence of malignancy at an earlier stage, allowing for the selection of more appropriate and potentially less aggressive therapeutic strategies (42, 43). The non-invasive nature of protein tumor markers, which can often be measured from blood or other easily obtainable samples, makes them an attractive option for widespread clinical use in lung cancer diagnostics. However, one of the major challenges with using these biomarkers for early detection is that the initial symptoms, including the rise in serum tumor markers, may be subtle or undetectable in the early stages of the disease (44). As a result,

many patients do not exhibit noticeable signs of the disease until it has progressed to a later stage, at which point the chances for successful treatment and survival are significantly reduced (45). Therefore, while biomarkers offer significant potential for early detection, their clinical application is still limited by the fact that early changes in tumor markers are often not immediately apparent, underscoring the need for improved methods to detect lung cancer at its earliest stages.

Serum markers, such as Carcinoembryonic antigen (CEA), cytokeratin 19 fragment (CYFRA21-1), and Neuron-specific enolase (NSE), have been widely studied for their potential role as prognostic or predictive factors in lung cancer (45). These biomarkers have shown promise in assessing disease progression, predicting patient outcomes, and guiding treatment strategies. However, as highlighted by Nagpal et al. (46), one of the main challenges in identifying effective biomarkers for cancer is that they are often present in very small amounts, especially during the early stages of cancer development. This makes it difficult to detect cancers at their earliest and most treatable stages, limiting the sensitivity of these markers as tools for early detection.

CEA was isolated and characterized in 1965 by Hao et al. as a colon cancer-specificantigen (47). It is elevated in many other cancers and benign diseases apart from colorectal cancer (47). Due to its elevated concertation in cancer tissue, CEA serves as a biomarker in clinical practice to determine the course of treatment and the impact of interventions on the health of the patients (48, 49). The specificity of this test in diagnosinglung cancer is rather low since the increased CEA may be found in other diseases as well. The elevated values of serum CEA are also reported in patients with lung fibrosis (50), pancreatic cancer, uterine, chronic obstructive pulmonary disease, Alzheimer's disease, rectal cancer, and lung cancer (47, 51).

Lack of differences in CEA concentrations in different conditions mean that there is a need for a more reliable biomarkers.

Another common marker employed in lung cancer diagnostics is CYFRA 21-1, which is part of the cytokeratin family. This protein is usually located in epithelial tissues,where it forms the filament cytoskeleton of epithelial cells (52). CYFRA 21-1 has been found valuable in NSCLC cases alongside other markers such as CEA or SCC antigen (53, 54). NSE is a glycolytic enzyme enolase isoenzyme markerfor neurons and peripheral neuroendocrine cells (55, 56). NSE has also been establishedas a useful biomarker for the diagnosis, staging, and management of SCLC (57). It is worth noting that NSE has also been found to be raised in NSCLC patients. Apart from its use in lung carcinoma, NSE is used in other NETs (58) and diseases such as neuroblastoma, melanoma, seminoma, renal cell carcinoma, Merkel cell tumor, carcinoid tumors, dysgerminomas, immature teratomas, Guillain-Barré syndrome, and Creutzfeldt-Jakob disease (59). Such broad disease diagnostic applications' spectrum indicates its low specificity.

Applying several biomarkers together may improve diagnostic efficacy. For example, CYFRA21-1 is useful for diagnosing NSCLC (60), especially SQCC and NSE iselevated in patients with small cell lung cancer SCLC and is useful for NETs (61). The incorporation of these biomarkers into a screening and diagnostic modeltogether with imaging and other diagnostic aids has the potential to enhance the early detection rates for NSCLC (62, 63). One of the reasons for the enhanced therapeutic efficacy is the ability to implement timely interventional measures. To that end, research studies like the one by Yang et al show that biomarkers coupled with evolving diagnostictechnologies enable the distinction between lung cancer and other respiratory diseases at an early stage, thereby improving treatment plans (64).

Differentiation of early stages of NSCLC is critical because it may help to asignificant proportion of lung cancer patients (65, 66). By using a complex diagnostic process and by application of markers like CEA, CYFRA21-1, and NSE, much can be understood about the early stages of lung cancer (67).

Another routinely used lung cancer marker is progastrin-releasing peptide (proGRP) which, after processing, may act as a neurotransmitter or neuromodulator in the brain or nervous system (68). Under normal physiological conditions, proGRP is synthesized as a precursor molecule and is cleaved off into its active end-product, GRP which is involved in the release of gastrin from the stomach. Recent studies have highlighted the significant role of the GRP and its receptor (GRPR) in inflammatory diseases, suggesting their potential as therapeutic targets. The GRP/GRPR signaling pathway has been implicated in various inflammatory conditions, including sepsis, asthma, rheumatoid arthritis, and inflammatory bowel disease (69). However, in the case of lung cancer, proGRP exists in a free form, and elevated concentrations of this molecule can be detected in the serum of SCLC patients (70). It has been established that proGRP has potential as a diagnostic and prognostic marker in SCLC (71). However, studies show that although moderately increased proGRP concentrations can be indicative of SCLC, they may also be linked to a non-malignant inflammation of the lungs (72). proGRP is synthesized when inflammation occurs in the pulmonary system and its synthesis is stimulated by immune cells like the macrophages and neutrophils, which release cytokines and chemokines (73). This indicates that there may be connections between pro-inflammatory cytokines and lung cancer, due to the ability of SCLC cells to produce proGRP in response to these signaling molecules.

Barchiesi et al. (74) demonstrated that proGRP serves as an effective diagnostic marker for distinguishing SCLC from NSCLC. The sensitivity and accuracy of proGRP were found to

be particularly significant in identifying SCLC patients, making it a valuable tool for early detection. Additionally, proGRP serum concentrations were shown to decrease following chemotherapy, underscoring its responsiveness to treatment and its potential for monitoring treatment outcomes. This highlights the importance of using biomarkers not only for accurate diagnosis but also for evaluating lung cancer status and assessing the effectiveness of therapeutic interventions (74, 75).

proGRP is a valuable biomarker for the early detection of SCLC, offering significant diagnostic potential by identifying malignant tumors at their initial stages. This early detection capability is crucial for improving patient prognosis, as timely diagnosis allows for more effective treatment options and better survival outcomes (76). However, it is important to note that low concentrations of proGRP are also observed in certain subgroups of NSCLC, which can complicate its use as a definitive marker for SCLC (61). Despite this, proGRP has demonstrated its utility in differentiating between various stages of NSCLC progression, providing clinicians with important information for monitoring disease advancement. By assisting in distinguishing between early and late-stage NSCLC, proGRP enhances the ability to tailor treatment strategies based on disease progression, thereby improving the overall management of patients with NSCLC (77). This underscores the versatility of proGRP as both a diagnostic and prognostic tool in lung cancer, highlighting its potential for broader clinical applications beyond just SCLC.

Begolli et al. identify proGRP as a promising biomarker for the diagnosis and prognosis of lung cancer, particularly in distinguishing early-stage adenocarcinoma (ADC) and squamous cell carcinoma (SQCC) from NETs, pneumonia, and COPD (78). Lower proGRP concentrations are associated with early-stage ADC and SQCC, while higher levels correlate

with NETs and inflammatory conditions, indicating its potential for early diagnosis and better differentiation of lung pathologies. However, the study also emphasizes challenges in interpreting proGRP levels, especially in inflammatory diseases like COPD exacerbations, where levels may overlap with those observed in early-stage small cell lung cancer (SCLC). This underscores the need for proGRP to be used in conjunction with other biomarkers for more accurate diagnosis. Despite these challenges, proGRP proves valuable in improving diagnostic workflows and patient stratification, particularly when integrated into multi-marker models, though further research is required to refine its clinical application and validate its effectiveness across diverse patient populations (78).

Currently, detecting marker alterations in early-stage cancers remains a challenge due to the complexity and sensitivity required for accurate diagnosis (79). However, ongoing research and advancements in technology are expected to significantly enhance marker analyses, leading to more precise and reliable detection methods. As our understanding of these biomarkers and their association with lung cancer continues to expand, it will pave the way for improved prevention strategies and more effective therapeutic approaches. These advancements have the potential to not only detect cancer at earlier, more treatable stages but also to personalize treatment regimens, ultimately improving patient outcomes (79). With more robust diagnostic tools, it may be possible to extend the life expectancies of patients and offer better quality of life by mitigating the impact of lung cancer and its complications. This highlights the importance of continued research and innovation in the field of cancer biomarkers for early detection and tailored treatment options.

In particular, early diagnosis of NSCLC is crucial as detection of the disease at the initial stage can increase the effectiveness of prescribed treatments. Still, further research is

needed to conclude the importance of alterations in proGRP concentrations in managing patients with early-stage NSCLC.

1.6. Carbohydrate Sulfotransferases as Candidate Biomarkers

Sulfotransferases (SULTs) are a diverse family of enzymes that catalyze the transfer of a sulfate group (SO_3) from 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to a variety of substrates, including hydroxyl and amine-containing molecules. This biochemical reaction, known as sulfation, is a critical cellular process that influences the structure and function of many biomolecules, including lipids, hormones, neurotransmitters, and xenobiotics. Sulfation generally enhances the solubility of compounds, making them easier for the body to excrete, and can also alter the biological activity of these molecules, affecting their interactions with receptors, enzymes, and other cellular components (80). Sulfation is an important set of processes that occurs in bacterial, plant and human cells and it plays an integral role in numerous cellular signaling events and alterations to receptor-ligand binding, including the action of hormones, chondrogenesis, cancer cell migration, and the detoxification of xenobiotics (81-83). There are two distinct categories of SULTs: soluble SULTs and integral SULTs membrane-bound SULTs or cellular SULTs. Membrane-bound SULTs are involved in the sulfation of tyrosine residues or carbohydrates that lead to the biosynthesis of glycoproteins, proteoglycans, and glycolipids, which play a critical role in numerous biological processes and disease states (83, 84). Carbohydrate sulfotransferases (CHST) have been known to catalyze the transfer of a sulfate group to particular carbohydrates (85). The CHST isoenzyme, pathway activity, substrate, product of the reported members of this family and classification by the name of the glycosaminoglycan (GAG) substrates

are summarized in Table 2 (86):

Table 2. Carbohydrate Sulfotransferase. (Source: created by the author of the dissertation)

Name (synonym)	Catalysed reaction
CHST1 (Keratansulfate Gal-6 sulfotransferase)	Sulphate transfer to position 6 of internal galactose (Gal) residues of keratan
CHST2 (N-acetylglucosamine 6-O-sulfotransferase 1)	Sulphate transfer to position 6 of non-reducing GlcNAc residues within keratan-like structures on N-linked glycans and within mucin-associated glycans
CHST3 (Chondroitin 6-0-Sulfotransferase 1)	Sulphate transfer to position 6 of GalNAc residue of chondroitin
CHST4 (Galactose/N-acetylglucosamine/N-acetylglucosamine 6-O-sulfotransferase 3)	Sulphate transfer to the hydroxyl group at C-6 position of the non-reducing GlcNAc residue within O-linked mucin-type glycans
CHST5 (N-acetylglucosamine 6-O-sulfotransferase 3)	Sulphate transfer to position 6 of non- GlcNAc residues and O-linked sugars of mucin-type acceptors
CHST6 (Corneal N-acetylglucosamine-6-O-sulfotransferase)	Sulphate transfer to position 6 of non-reducing GlcNAc residues of keratan
CHST7 (Chondroitin 6-sulfotransferase 2)	Sulphate transfer to position 6 of non- GlcNAc residues and to position 6 of the GalNAc residue of chondroitin
CHST8 (GalNAc-4-O-sulfotransferase 1)	Sulphate transfer to position 4 of non-reducing GalNAc residues in both N-glycans and O-glycans
CHST9 (GalNAc-4-O-sulfotransferase 2)	Sulphate transfer to position 4 of non-reducing GalNAc residues in both N-glycans and O-glycans
CHST10 (Human Natural Killer-1 sulfotransferase)	Sulphate transfer to position 3 of terminal glucuronic acid of both protein- and lipid-linked oligosaccharides
CHST11 (Chondroitin 4-O-sulfotransferase 1)	Sulphate transfer to position 4 of GalNAc residue of chondroitin
CHST12 (Chondroitin 4-O-sulfotransferase 2)	Sulphate transfer to position 4 of GalNAc residue of chondroitin and desulfated dermatan sulphate
CHST13 (Chondroitin 4-O-sulfotransferase 3)	Sulphate transfer to position 4 of GalNAc residue of chondroitin
CHST14 (Dermatan 4-sulfotransferase 1)	Sulphate transfer to position 4 of GalNAc residue of dermatan sulphate
CHST15 (N-acetylgalactosamine 4-sulphate 6-O-sulfotransferase/B-cell recombination-activating genes-associated gene protein)	Sulphate transfer to C-6 hydroxyl group of the GalNAc 4-sulfate residue of chondroitin sulphate A and forms chondroitin sulphate E containing GlcA-GalNAc(4,6-SO_4) repeating units

CHST catalyze the transfer of sulfate groups to certain carbohydrates. These enzymes have a role in facilitating crucial extracellular signaling mechanisms in both health and disease. They participate in pathological conditions, such as cancer and theyhave been considered for both diagnostic and prognostic purposes (87). CHSTs are crucial in modifying the sulfation patterns of GAGs, which significantly impacts their interactions with biological

molecules like growth factors, cytokines, and cell surface receptors. This sulfation plays a vital role in regulating key cellular processes such as signaling, adhesion, migration, and tissue organization, all of which are essential for maintaining normal physiological functions. In disease states, alterations in CHST activity or expression can disrupt these processes, leading to pathological conditions. For example, in connective tissue disorders like skeletal dysplasia and chondrodysplasia, changes in CHST function affect the synthesis and function of GAGs, resulting in structural abnormalities in bone and cartilage. Similarly, in cancer, CHSTs have been implicated in tumor progression and metastasis, with overexpression of certain CHST enzymes linked to poor prognosis. In malignancies such as ovarian, pancreatic, and glioblastoma cancers, increased sulfation of GAGs can promote tumor cell proliferation, invasion, and resistance to therapy (86).

CHST catalyze the transfer of sulfate groups to certain carbohydrates. These enzymes have a role in facilitating crucial extracellular signaling mechanisms in both health and disease. They participate in pathological conditions, such as cancer and they have been considered for both diagnostic and prognostic purposes (87). Beyond cancer, CHSTs are emerging as key players in inflammation, where their regulation of GAG sulfation affects immune cell recruitment, activation, and the regulation of inflammatory mediators. In diseases like rheumatoid arthritis and inflammatory bowel disease, changes in CHST activity can contribute to altered tissue remodeling and immune cell trafficking. Recent studies have also shown that CHST11 and CHST15 overexpression in vascular smooth muscle cells is associated with severe lung pathology in COVID-19 patients, suggesting that CHSTs may play a role in viral-induced inflammation and tissue damage. As research into the role of CHSTs in disease advances, these enzymes hold significant promise as diagnostic and

prognostic biomarkers, as well as therapeutic targets for a range of diseases (86). Their potential for early detection and disease monitoring, coupled with their involvement in critical cellular processes, makes CHSTs a promising area for further clinical investigation (88-90)

Different categories of CHSTs have been associated with the development of cancer and cancer growth (91). For example, CHST7, CHST11, CHST12, CHST13, andCHST15 have shown functional value and predicting ability in different kinds of cancer (92-94). These enzymes are involved in post-translational modifications involved in cell signaling and growth control, and they are widely implicated in cancer. Other modifications such as DNA methylation of the *CHST7* gene play a crucial role intumorigenesis (95). *CHST7* gene was found to be hypermethylated in pituitary adenomasand hypermethylation of this gene was found to be related to increased tumor proliferation(95). Similar association was detected in colorectal cancer wherein it was determined thathypermethylated *CHST7* in white blood cells elevated the probability of colorectal cancerdiagnosis (95). The role of CHST7 has been further affirmed in the differentiation of non-malignant pulmonary inflammations and lung cancer (88). This study revealed differentialexpression of the CHST7 protein in inflammation and cancer which suggests that CHST7could be used for differentiation of lung cancer from the acute lung inflammations. CHST7is implicated in the modulation of chondroitin sulfate proteoglycan (CSPG) which is implicated in metastatic processes and carcinogenesis, hence its value in cancer distinction from inflammation (95). Thus, gaining a more comprehensive understanding of CHST7, particularly its role in the regulation of CSPGs, as well as its influence on cancer metastasis and inflammatory processes, could pave the way for the development of more advanced and effective diagnostic tools. These insights highlight the potential significance of CHST7, not only within the specific context of lung

cancer but also more broadly across the fields of oncology and inflammation. By exploring the mechanisms through which CHST7 contributes to these pathological processes, researchers may uncover novel applications for this molecule, solidifying its relevance as a biomarker. Such observations underscore the promise of CHST7 as a valuable candidate for further investigation, particularly in the pursuit of enhanced diagnostic accuracy and the early detection of lung cancer. Furthermore, its dual involvement in both cancer progression and inflammatory responses positions CHST7 as a potential key player in the interface between tumor biology and immune regulation, offering opportunities for future translational research and clinical applications (95).

1.7. The Study Rationale

Application of serum protein markers in a clinical setting may improve the managing of lung cancer. This study deals with the need to enhance the diagnostic capability to differentiate between early-stage pulmonary cancers and COPD and other benign pulmonary inflammations. A critical analysis of the available literature shows that standard serum markers include CEA, CYFRA21-1, NSE, and proGRP have low specificity and sensitivity as markers for early lung cancer detection (48). This dissertation seeks to overcome these limitations by introduction of CHST7 as a new biomarker in inflammation and lung cancer and evaluation of proGRP diagnostic properties in inflammation and NSCLC.

This will be achieved through patient enrollment using the inclusion and exclusion criteria to reach an appropriate subject pool. The study will compare serum proGRP and CHST7 diagnostic properties with properties of routinely used markers (CEA, CYFRA21-1, and NSE) by observational case-control approach. This comparison is necessary to conclude whether CHST7 and proGRP may differentiate lung cancer in its early stages from the pulmonary inflammations more reliably than the existing markers. Increased diagnostic accuracy in the early detection and differentiation of pulmonary malignancies from benign conditions, if achieved, may lead to better therapy planning and prognosis.

1.8. Problem Statement

Lung cancer and COPD are two of the most significant health issues that affect a large portion of the population worldwide and are also characterized by high mortality rates (6, 12). Lung cancer screening and detection at a very early stage may increase the chances of the patient's survival. However, the current diagnostic techniques are limited in detecting early-stage lung cancer from COPD and other non-malignant diseases of the lungs (96). Serum markers such as CEA, CYFRA21-1, NSE, and proGRP have moderate-to-low diagnostic sensitivity and specificity (47). Thus, it is crucially important to develop biomarkers that would help better define malignant and non-malignant pulmonary diseases at early stages.

Diagnostic properties of markers like CHST7 and proGRP appear to be promising. Initial investigations show that CHST7 has potential as a biomarker of pulmonary inflammation and lung cancer (88, 95) but there is a need for extensive research to evaluate these markers and to compare them to already existing markers: diagnostic performance of CHST7 and proGRP will be compared to the performance of CEA, CYFRA21-1 and NSE in differentiation of early-stage NSCLC from COPD and other benign pulmonary inflammations. This study is carried out to advance other diagnostic approaches to contribute to a better decision-making process of diagnosis, therefore increasing the chances of patients' survival.

2. HYPOTHESES

1. Serum proGRP and CHST7 are associated with the early stages of lung cancer and non-malignant lung inflammations.

2. proGRP and CHST7 enable differentiation of patients with the early stages of lung cancer from patients suffering from non-malignant lung inflammations.

3. OBJECTIVES

1. Assess statistical association between proGRP and CHST7 with early stages of lung cancer and non-malignant pulmonary inflammations.

2. Evaluate diagnostic accuracy of CEA, CYFRA and NSE in differentiation of early stages of lung cancer from COPD and benign pulmonary inflammations.

3. Assess diagnostic value of the potential marker proGRP and CHST7 in differentiation between early stages of lung cancer and non-malignant pulmonary inflammations.

4. MATERIALS AND METHODS

4.1. Study design

A clinical case-control study was designed to assess the potential association between proGRP serum levels, inflammation, and early-stage (stage 1 and 2) lung cancer which was carried outbetween 2020 and 2022 at the Clinical Hospital Centre Zagreb, Croatia, and Osijek University Hospital Centre, Croatia. The study was open to male and female patients whosigned an information disclosure consent and were admitted to the hospital due to the suspected malignant lung illness. Both, clinical and laboratory evidence of inflammation and lung cancer were collected. Patients were included based on physical and radiological (chest x-ray) examinations. Pulmonary inflammation was diagnosed according to the National Institute for Health and Care Excellence (97) guidelines. The diagnosis of COPD patients was established according to the updated version of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) (98) practice guidelines. The cancer diagnosis was established based on diagnostic procedures such as a chest x-ray, CT scan and lung needle biopsy in accordance with the World Health Organization(WHO) classification of lung tumors (99). The clinical staging of malignant cases was done using the TNM staging approach.

The case-control design is justified as practical and feasible because of investigating a relatively low-incidence disease, such as early-stage lung cancer. It determines diagnostic marker properties based on the presence or absence of the disease in question. This design also allows testing several markers at once, which enables a better understanding of the differences in diagnostic potential of the biomarkers

in question. Moreover, the staff-patient ratio is higher in clinical centers, which makes the assessment measures and data collection more accurate and objective.

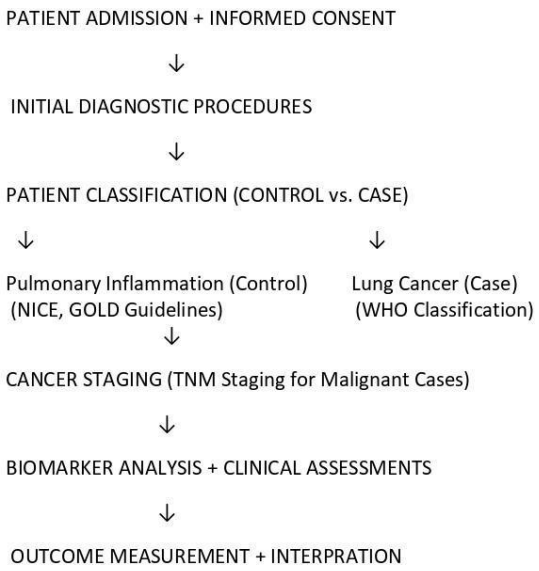

Figure 2. Flowchart for research methodology. (Source: created by the author of the dissertation)

4.2. Participants

Participants in this study have been recruited based on inclusion and exclusion criteria (Table 3). Sampling size of N = 198 was chosen to fit the need for proper statistical assessment of differences and associations. Participants were early-stage lung cancer patients (stage I or II) and patients with non-malignant pulmonary inflammation, COPD and acute inflammation, in particular. Vein blood was drawn from all participants to determine the CHST7, proGRP, CEA, NSE, CYFRA 21-1, and C-reactive protein (CRP) concentrations.

Table 3: Inclusion and exclusion criteria. (Source: created by the author of the dissertation)

Inclusion Criteria
Age > 18 years
Diagnosis of lung cancer or non-malignant pulmonary inflammation
Tumor stage I or II
Exclusion Criteria
Therapeutic Treatment
Presence of secondary tumors
Tumor stage III and IV
Incomplete medical documentation

4.3. Analytical methods

Blood samples were collected from subjects following standard laboratory procedures to ensure safety and minimize any discomfort during the blood draw. The collection process was carefully performed to adhere to ethical guidelines and reduce the risk of complications. For the purpose of serum extraction, blood was drawn into tubes that did not contain anticoagulants (Becton Dickinson, Plymouth, UK). Following collection, the tubes were promptly transported to the laboratory, where they were centrifuged at 1300 g for 10 minutes within an hour of collection to separate the serum from the blood cells. This centrifugation step was essential for obtaining clear serum that could be further analyzed. Once the serum was separated, it was aliquoted into smaller portions to prevent multiple freeze-thaw cycles, which can affect sample integrity. These aliquots were then placed into a freezer maintained at a temperature of -70°C for long-term storage. This freezing process ensures that the serum samples remain stable and suitable for future measurements, maintaining their quality for accurate analysis when required.

CEA, CYFRA 21-1, and proGRP were measured on a COBAS E 601 analyzer (Roche Diagnostics, IN, USA) using electrochemiluminescence immunoassay (ECLIA) technique. CHST7 was assayed by enzyme-linked immunosorbent assay (ELISA) method (Cloud-Cloun corp., Wuhan, China) using the ETI-Max 3000 (DiaSorin Saluggia Italy) analyzer. CRP concentration was determined by immunoturbidimetry on AU680 automatic chemistry analyzer (Beckman-Coulter, USA). All assays were verified by the manufacturers and internal and external quality control measures were regularly performed, making the results collected coherent and comprehensive. These stringent measures that would be observed

and followed in the process of sample collection and analysis produced reliable data suitable for assessments of markers in differentiation of the early-stage lung cancer from non-malignant pulmonary inflammations.

4.3.1. CHST7 measurement

The microplate was initially pre-coated with a specific CHST7 antibody to facilitate the detection of CHST7 in the samples. Standards and experimental samples were then added to the wells of the microplate. To ensure proper binding, each well was subsequently treated with a biotin-conjugated CHST7 antibody, which targeted the CHST7 protein. Following this, an avidin-horseradish peroxidase (HRP) complex was introduced into each well. The avidin component of the complex specifically bound to the biotin-conjugated antibody, forming a stable complex. After a period of incubation, the HRP enzyme catalyzed a reaction with its substrate, resulting in a color change in the wells. To halt the enzyme-substrate reaction at the appropriate point, sulphuric acid was added. The resulting color change was quantitatively measured using a spectrophotometer set to 450 nm ± 10 nm. Finally, the optical density (O.D.) of each sample was compared to a pre-established standard curve, which enabled the determination of CHST7 concentration in the samples. This method allows for precise and reliable quantification of CHST7 levels, facilitating its use in diagnostic or research applications.

4.3.2. CEA, CYFRA 21-1, proGRP and NSE Determination

Serum samples were incubated with antibodies that were specifically designed to recognize and bind to the target antigens: CEA, CYFRA 21-1, proGRP, and NSE. During this incubation process, if the corresponding antigens were present in the serum samples, they would interact with and bind to the labeled antibodies, resulting in the formation of antigen-antibody complexes. These complexes are crucial for the subsequent detection and quantification of the specific antigens. To facilitate the detection process, an electrical potential was applied to the sample, which activated the electrochemiluminescent tag attached to the antibody. Upon activation, the electrochemiluminescent tag emitted light and the intensity of the emitted light was then measured and quantified. This light emission is directly proportional to the concentration of the specific antigen (CEA, CYFRA 21-1, proGRP, or NSE) present in the serum sample. By measuring the light intensity, the concentration of each antigen can be accurately determined, providing valuable information for diagnostic purposes.

4.3.3. CRP measurement

The serum samples were mixed with a reagent containing specific antibodies targeting C-reactive protein (CRP). Upon binding to CRP, these antibodies form stable antigen-antibody complexes that are insoluble in the solution, resulting in increased turbidity. The level of turbidity, which corresponds to the amount of light scattering in the sample, is directly related to the concentration of CRP present. By measuring the light transmission through the sample, the degree of scattering can be quantified, providing an accurate determination of CRP concentration. The higher the CRP concentration, the greater the turbidity, ensuring precise measurement of CRP levels.

4.4. Statistical Methods

The R programming environment ver. 4.2.0. and MedCalc software system (version 22.014, MedCalc Software Ltd, Ostend, Belgium) were used for all calculations (40). The Mann-Whitney U (MWU) test was used to compare serum tumor marker concentrations coming from the different patient groups. Correlations were determined using Pearson correlation coefficient. As a part of with receiver operating characteristic (ROC) analysis, the difference in ROC area-under-curve (AUC), i.e. Δ ROC AUC, has been used in this study (41). *P*-values < 0.050 were considered statistically significant.

5. RESULTS

5.1 Demographics

The research included a total of 198 patients: 107 with early-stage lung cancer and 91 with inflammation, consisting of men and women aged 43 to 90 years. Gender distribution differed between the early-stage lung cancer and inflammation groups. Among patients with early-stage lung cancer, there were significantly more men than women, as was the case in the inflammation group. The median age of patients with early-stage lung cancer and inflammation also differed slightly. For those with early-stage lung cancer, the median age was 63 years, while for those with inflammation, it was 70 years (Table 4).

Table 4. Demographic and clinical data

Properties/Cases	**Early-stage lung Cancer**	**Inflammations**
n =	107	91
Habits		
Smoker	34	15
Ex-Smoker	51	44
Nonsmoker	22	31
Lung Cancer Histology		
ADC	67	
SQCC	27	
Large cell lung carcinoma (LCLC)	2	
Small cell lung carcinoma (SCLC)	3	
Carcinoid	8	
Cancer Stage		
Stage I	54	
Stage II	53	
Inflammation		
Pneumonia	-	25
COPD-Exacerbation (COPD_E)	-	14
COPD-Remission (COPD_R)	-	52
Gender		
Male	79	68
Female	28	23
Age (Years)		
Median (range)	63 (43 – 78)	70 (47 - 90)

5.2. Initial observations

Based on the provided marker concentrations for CHST7, CEA, proGRP, CYFRA 21-1, and NSE across patients with early-stage lung cancer, COPD, and pneumonia, the data suggests varying degrees of diagnostic utility for each marker in distinguishing between these conditions. The concentration levels of CHST7 were found to be highest in patients diagnosed with pneumonia, indicating its potential role in inflammation and infection. In contrast, CHST7 concentrations were the lowest in individuals with early-stage lung cancer, which may suggest a lesser involvement of this marker in cancerous processes at initial stages. This differential distribution of CHST7 levels could reflect its possible association with the inflammatory response that is more pronounced in pneumonia, compared to the more complex pathophysiology of lung cancer.

CEA concentrations, on the other hand, were observed to be highest in early-stage lung cancer patients, where they significantly exceeded the reference ranges (100-104) (Table 5).

Table 5. Serum reference range CEA, proGRP, CYFRA 21-1, NSE and CRP

Biomarkers	**CEA** (µg/L)	**proGRP** (ng/L)	**NSE** (µg/L)	**CYFRA21-1** (µg/L)	**CRP** mg/L
Reference range	Nonsmokers < 3.4 Smoker < 4.3	63.7 – 74.5	< 16.3	< 3.5	< 5.0

The serum reference ranges for carcinoembryonic antigen (CEA), pro gastrin releasing peptide (proGRP), cytokeratin 19 fragment (CYFRA 21-1), neuron specific enolase (NSE), and c-reactive protein (CRP) are established to help identify and monitor various conditions, particularly in cancer and inflammation. These biomarkers have specific reference intervals that aid in diagnosing and assessing the severity of diseases such as lung cancer and inflammatory disorders. (Source: created by the author of the dissertation)

Table 6 represents the median concentrations of five different biomarkers (CHST7, CEA, proGRP, CYFRA 21-1, and NSE) across three groups: patients with early-stage lung cancer, patients with COPD, and patients with pneumonia. Concentrations of five biomarkers (CHST7, CEA, proGRP, CYFRA 21-1, and NSE) were slightly elevated in COPD patients and within the normal range for pneumonia patients. Median proGRP was the highest in pneumonia patients and the lowest in early-stage lung cancer patients. COPD patients fall within the reference range. proGRP concentrations in early-stage lung cancer were lower compared to pneumonia. CYFRA 21-1 concentrations were within the reference range for early-stage lung cancer and COPD patients. Pneumonia patients showed slightly elevated concentrations, exceeding the reference range. CYFRA 21-1 was more associated with pneumonia in this dataset. While NSE showed a trend towards higher concentrations in the early-stage lung cancer patients, its values remain within the reference range, suggesting that it cannot be used for the early lung cancer detection.

Table 6. Serum concentrations of CHST7, CEA, proGRP, CYFRA 21-1, and NSE across three groups of patients

	CHST7 (µg/L)	**CEA** (µg/L)	**proGRP** (ng/L)	**CYFRA 21-1** (µg/L)	**NSE** (µg/L)
Ca Stage 1 and 2	73,4 (38.2)	8,6 (3.4)	51,8 (18.2)	3,0 (1.6)	12,9 (3.9)
COPD	131,0 (89.6)	4,4 (4.2)	63,3 (19.7)	2,1 (0.9)	10,3 (3.7)
Pneumonia	263,1 (61.7)	3,5 (3.1)	94,9 (59.1)	3,6 (2.1)	5,0 (3.0)

Data are presented as median and interquartile range (IQR) of serum concentrations for carbohydrate sulfotransferase 7 (CHST7), carcinoembryonic antigen (CEA), pro gastrin releasing peptide (proGRP), cytokeratin 19 fragment (CYFRA 21-1) and neuron specific enolase (NSE) in three groups of patients.

5.3. In-depth proGRP analysis

Box-and-whisker plots showing serum proGRP distributions in lung cancer and non-malignant pulmonary inflammations are given in Figure 3. Differences in the serum proGRP concentrations in different types of non-malignant lung inflammations and different types of early lung cancer were statistically significant (n = 198, $P < 0.001$), indicating an appreciable difference between proGRP values between cancer and non-malignant lung inflammation.

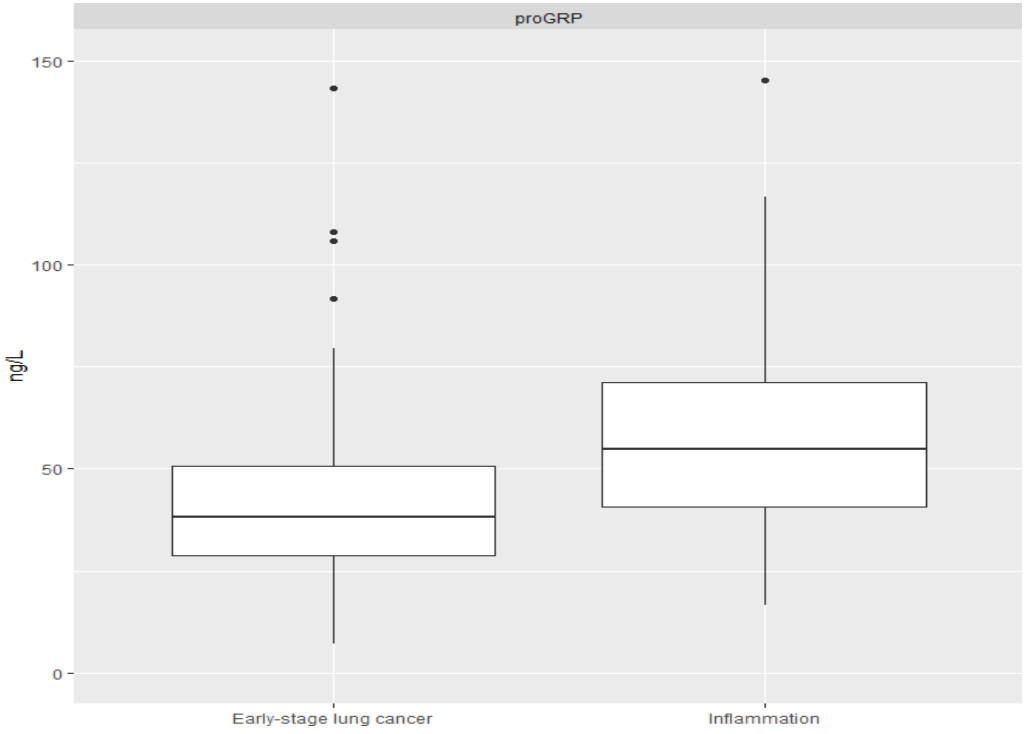

Figure 3. Serum pro gastrin releasing peptide (proGRP) distributions in lung cancer and non-malignant pulmonary inflammations. X axis represent pathological states while Y axis represent pro gastrin releasing peptide (proGRP) concentration. The statistical analysis using the Mann-Whitney U test provides insight into the difference between the two groups.

In the group of early-stage lung cancer, the concentrations of proGRP were significantly decreased, with the median and the upper quartile was higher in inflammation patients: only the outliers that correspond to SCLC and other NETs did not follow this description. These differences in proGRP concentration imply that proGRP may be used to distinguish between early-stage NSCLC, comprising the majority of enrolled patients, and benign pulmonary inflammations.

In Figure 4. proGRP concentrations were compared across a variety of lung conditions and cancer types, including COPD remission (COPD_R), COPD exacerbation (COPD_E), Pneumonia, ADC, SQCC, Carcinoid, LCLC, and SCLC, using the Box and Whisker plot. proGRP concentrations in ADC and SQCC were low and exhibited limited variability, suggesting that these cancers do not cause a substantial increase of proGRP. COPD, particularly during exacerbations, pneumonia, and carcinoid tumors exhibited moderately elevated proGRP concentrations with noticeable variability. The median proGRP concentrations in LCLC and SCLC were found to be high, with SCLC exhibiting the highest concentrations and the largest variability.

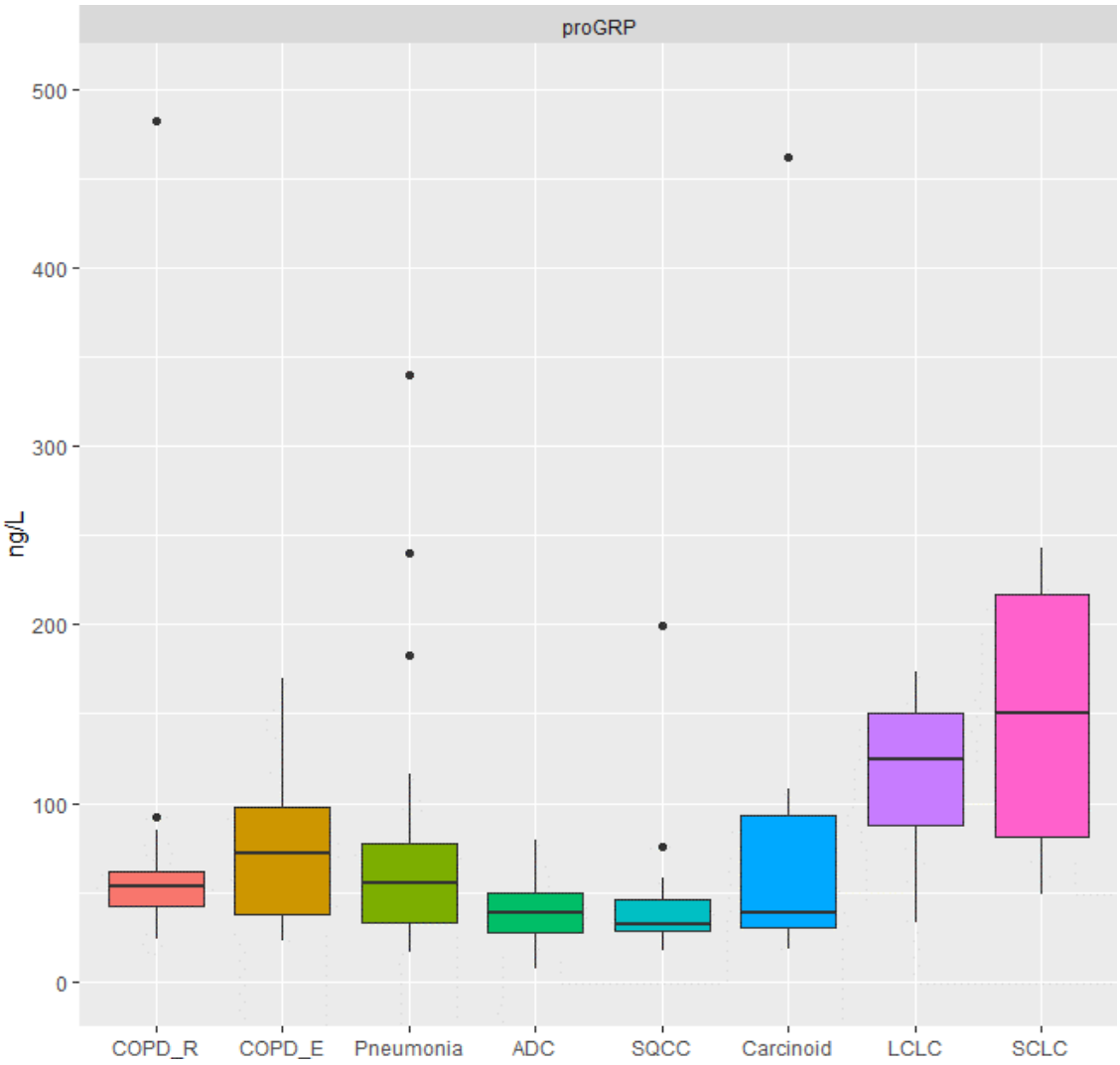

Figure 4. Serum pro gastrin releasing peptide (proGRP) in different types of non-malignant lung inflammations and different types of early lung cancer, chronic obstructive pulmonary diseases remission (COPD_R, N=52), chronic obstructive pulmonary diseases exacerbation (COPD_E, N=14), pneumonia (N=25), adenocarcinoma (ADC, N=67), squamous cell carcinoma (SQCC, N=27), carcinoid (N=8), large cell lung carcinoma (LCLC, N=2), small cell lung carcinoma (SCLC, N=3). X axis represent pathological states while Y axis represent proGRP concentration. Median and IQR (Interquartile Range) used to assess central tendency and spread of data in each group.

A Pearson correlation analysis was performed to investigate the connection between the concentrations of proGRP and CRP (Figure 5).

The analysis revealed that there was a statistically significant positive correlation with a Pearson correlation coefficient (r) of 0.241 ($P < 0.001$). This positive correlation suggests that as CRP concentrations increased, proGRP concentrations also tend to increase, and *vice versa*. However, the magnitude of the correlation coefficient indicated that changes in CRP concentrations explain only a limited amount of the variability observed in proGRP concentrations.

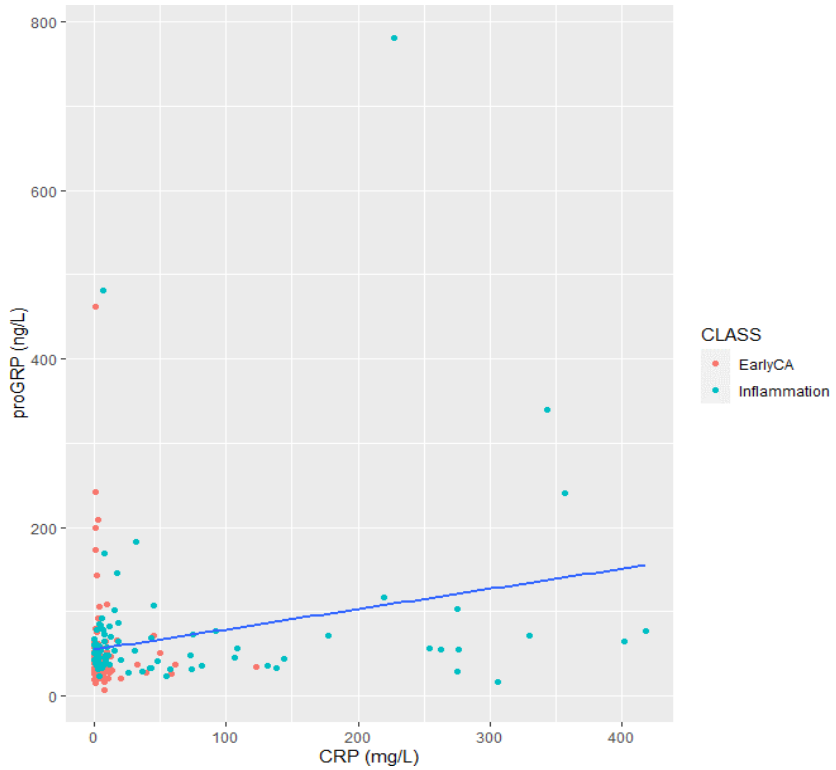

Figure 5. Serum pro gastrin realizing peptide (proGRP) and c reactive protein (CRP) relationship in early-stage lung carcinoma and inflammations (N = 197). Pearson correlation was used to assess the strength and direction of the linear relationship between variables, while linear regression was applied to model and quantify the relationship.

A correlation study was conducted to determine the extent to which proGRP is associatedwith inflammation and the extent to which CRP is associated with NSE. The latter was done due to the fact that proGRP and NSE alike are used in NET/SCLC diagnostics. Theanalysis of the relationship between NSE and proGRP revealed a statistically significantnegative correlation (Figure 6). The Pearson correlation coefficient (r) was -0.177 (P = 0.031). This negative correlation suggests that as NSE concentrations increase, proGRPconcentrations tend to decrease. However, the coefficient's proximity to zero indicates aweak negative linear relationship, implying that changes in NSE concentrations cannot explain the variability in proGRP concentrations. Although proGRP and NSE are both established biomarkers for SCLC and NET, the study revealed that they behave quite differently. This suggests that while both markers are useful in the diagnosis andmonitoring of these cancers, they may reflect different aspects of tumor biology or respond differently to various clinical conditions, such as inflammation. The differing behavior of proGRP and NSE emphasizes the importance of considering both markers in a complementary manner when assessing patients with SCLC or NET.

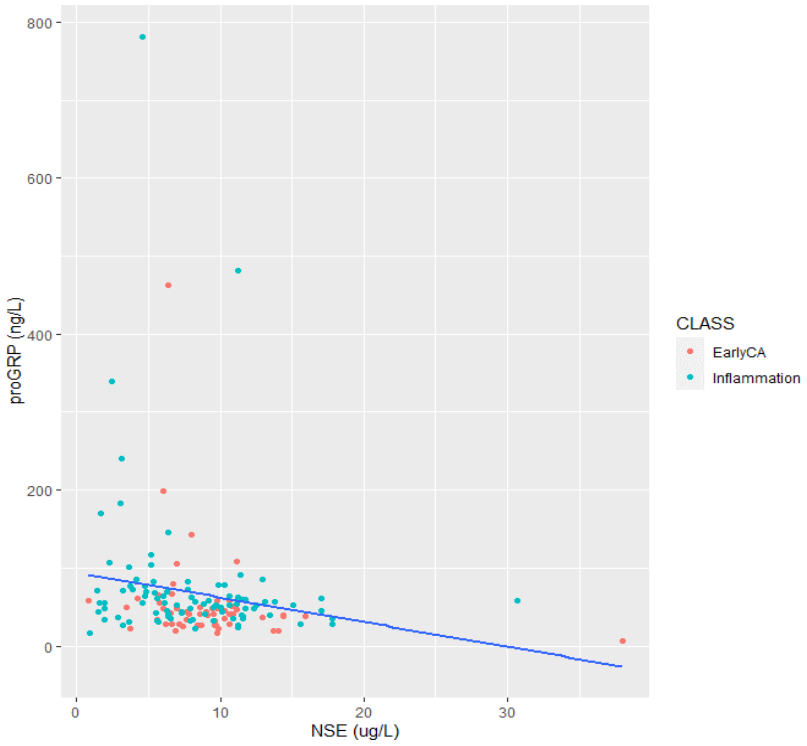

Figure 6. Serum pro gastrin releasing peptide (proGRP) and neuron specific enolase (NSE) relationship in early-stage lung carcinoma and inflammation (N=149). Pearson correlation was used to assess the strength and direction of the linear relationship between variables, while linear regression was applied to model and quantify the relationship.

The correlation analysis within the carcinoma population was conducted to determine whether inflammation, as indicated by elevated CRP concentrations, significantly increases proGRP concentrations in patients with carcinoma. The Pearson correlation coefficient (Figure 7) between proGRP and CRP in early-stage lung carcinoma (> 5 mg/L), was 0.064 (P = 0.720). P value suggests that there was no correlation between the serum proGRP and CRP in patients having inflammation accompanied by the early-stage lung cancer. This is an indication that CRP alone cannot differentiate between only inflammation and inflammation accompanied by early-stage lung cancer.

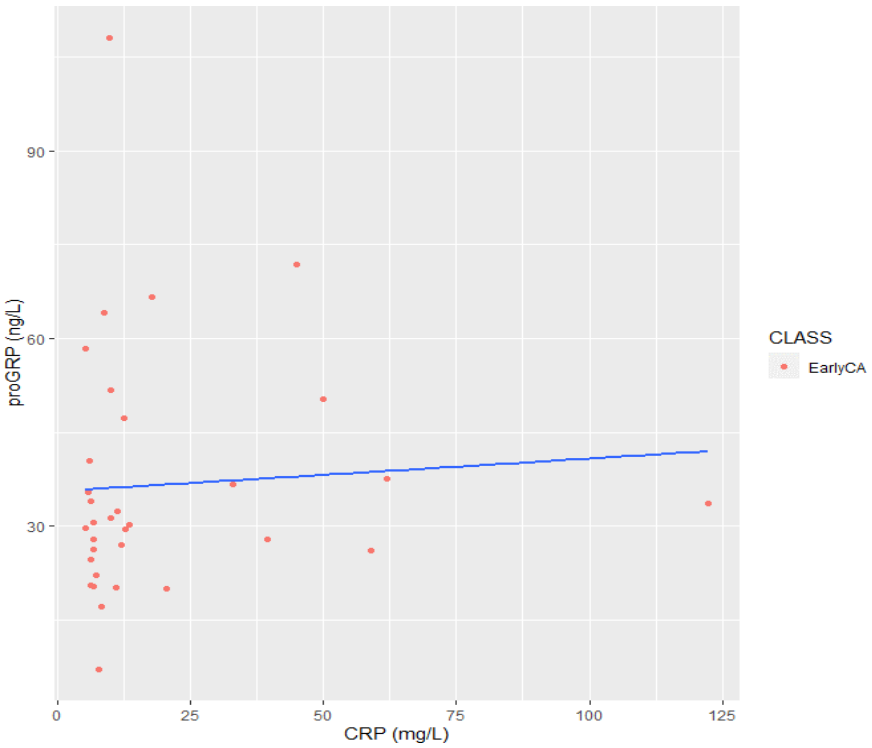

Figure 7. Serum pro gastrin releasing peptide (proGRP) and positive c reactive protein (CRP > 5 mg/L) relationship in early-stage lungcarcinoma and inflammation (N = 33). Pearson correlation was used to assess the strength and direction of the linear relationship between variables, while linear regression was applied to model and quantify the relationship.

5.4. In-depth CHST7 analysis

According to the manufacturer's instructions, we obtained measurable values for CHST7 controls in two occasions. The first measurement was 1.28 µg/L, and the second measurement was 132.3 µg/L. These values were significantly lower than the targeted values of 1211.1 µg/L and 3381.1 µg/L, respectively. Therefore, it is recommended to utilize more reliable methods to measure this parameter.

The Box and Whisker plot (Figure 8) illustrates the distribution of serum CHST7 concentrations in patients with early-stage lung cancer compared to those with inflammation. The boxplot analysis revealed significant differences in CHST7 concentrations between patients with early-stage lung cancer and those with inflammation. The data suggests that CHST7 concentrations were generally higher and more variable in inflammatory conditions compared to early-stage lung cancer.

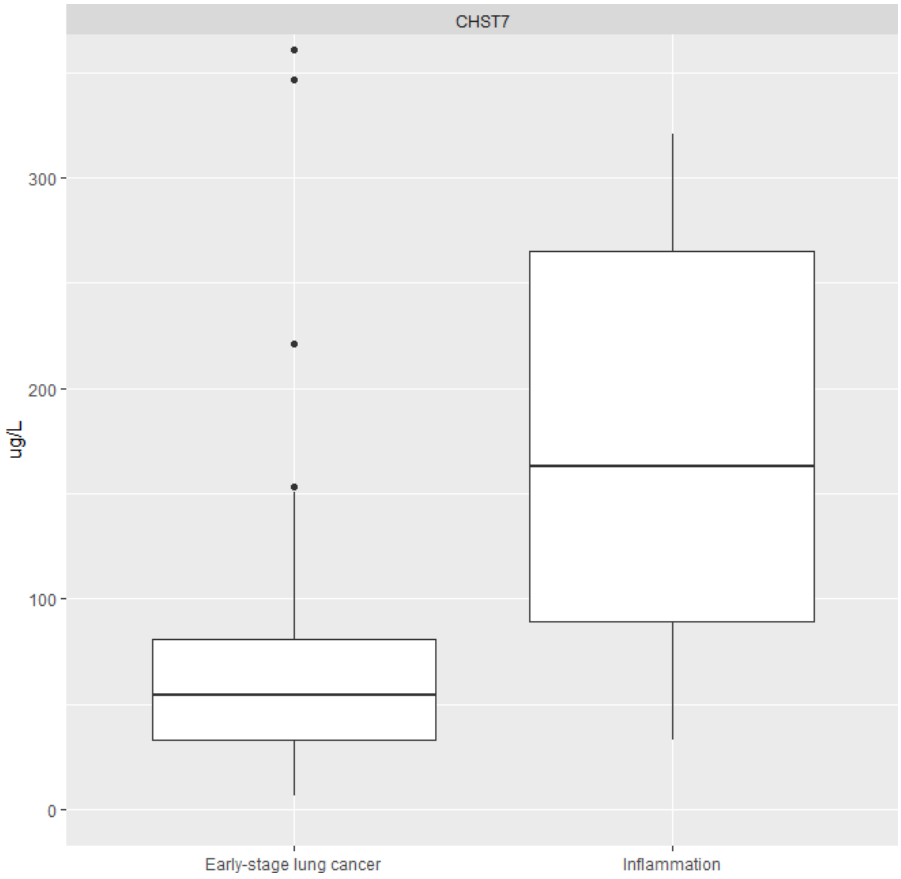

Figure 8. Box and Whisker plots. Carbohydrate sulfotransferase 7 (CHST7) in early lung cancer and non-malignant lung inflammations. X axis represent pathological states while Y axis represent CHST7 concentration. The statistical analysis using the Mann-Whitney U test provides insight into the difference between the two groups.

In Figure 9 CHST7 was compared across several lung diseases and types of cancer, including COPD_R, COPD_E, Pneumonia, ADC, SQCC, Carcinoid, LCLC, and SCLC, using a Box and Whisker plot. The distributions of COPD_R and COPD_E were similar, with median concentrations slightly exceeding 250 µg/L. The concentrations of expression in cancer groups (ADC, SQCC, Carcinoid, LCLC, and SCLC) tend to be lower compared to those in COPD and pneumonia. Pneumonia exhibits elevated CHST7 concentrations in comparison to other COPD, with a median concentration of around 300 µg/L and a wider interquartile range, suggesting greater variability. ADC, SQCC, LCLC, and SCLC have decreased and less fluctuating concentrations of CHST7, with median values below 100 µg/L, suggesting comparatively lower concertation of CHST7.

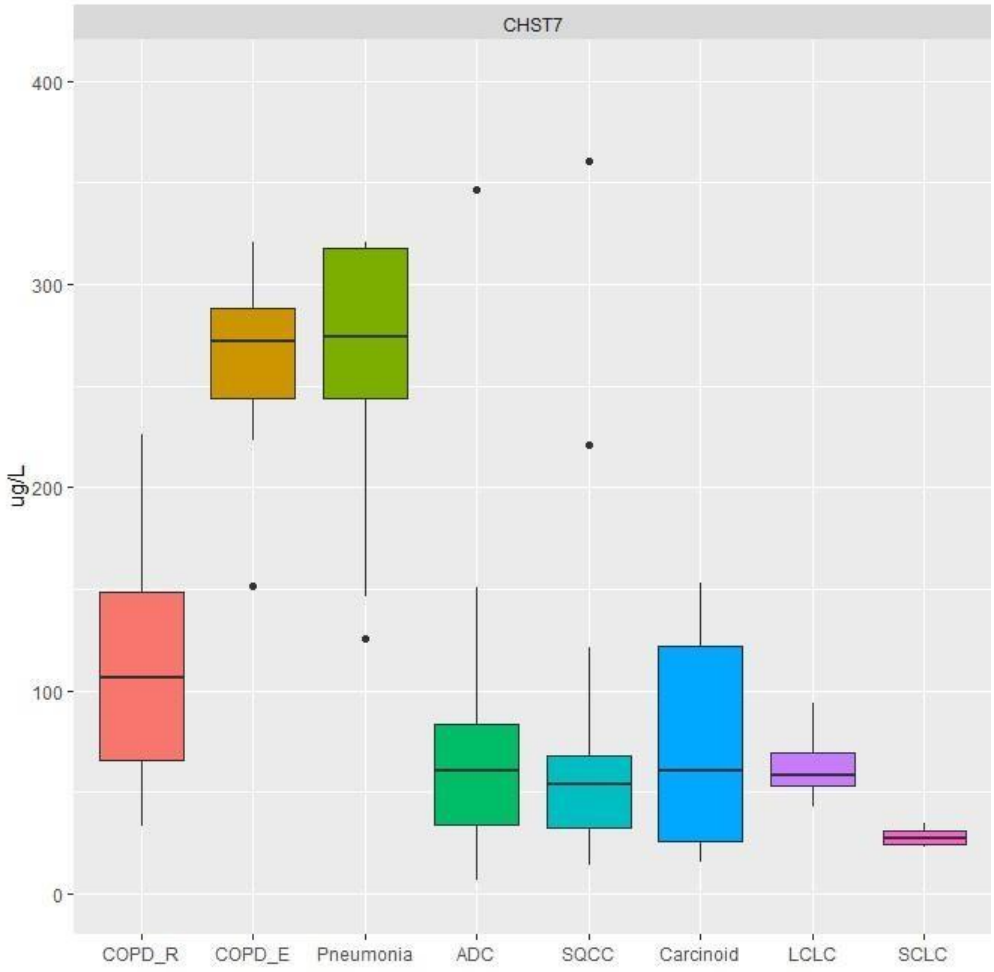

Figure 9. Carbohydrate sulfotransferase 7 (CHST7) in different types of non-malignant lung inflammations and different types of early lung cancer, chronic obstructive pulmonary diseases remission (COPD_R, N=52), chronic obstructive pulmonary diseases exacerbation (COPD_E, N=14), pneumonia (N=25), adenocarcinoma (ADC, N=67), squamous cell carcinoma (SQCC, N=27), carcinoid (N=8), large cell lung carcinoma (LCLC, N=2), small cell lung carcinoma (SCLC, N=3). X axis represent pathological states while Y axis represent CHST7 concentration. Median and IQR (Interquartile Range) used to assess central tendency and spread of data in each group.

A Pearson correlation analysis was performed to evaluate the relationship between serum CHST7 and NSE concentrations, as shown in Figure 10. The analysis revealed a correlation coefficient of r=−0.314, with a statistically significant p-value of $P < 0.001$. This indicates a moderately inverse relationship between the two variables, suggesting that higher serum NSE concentrations were generally associated with lower serum CHST7 concentrations in the studied population. This negative correlation points to a potential interaction or interplay between these biomarkers, which may reflect underlying biological mechanisms influencing their levels.

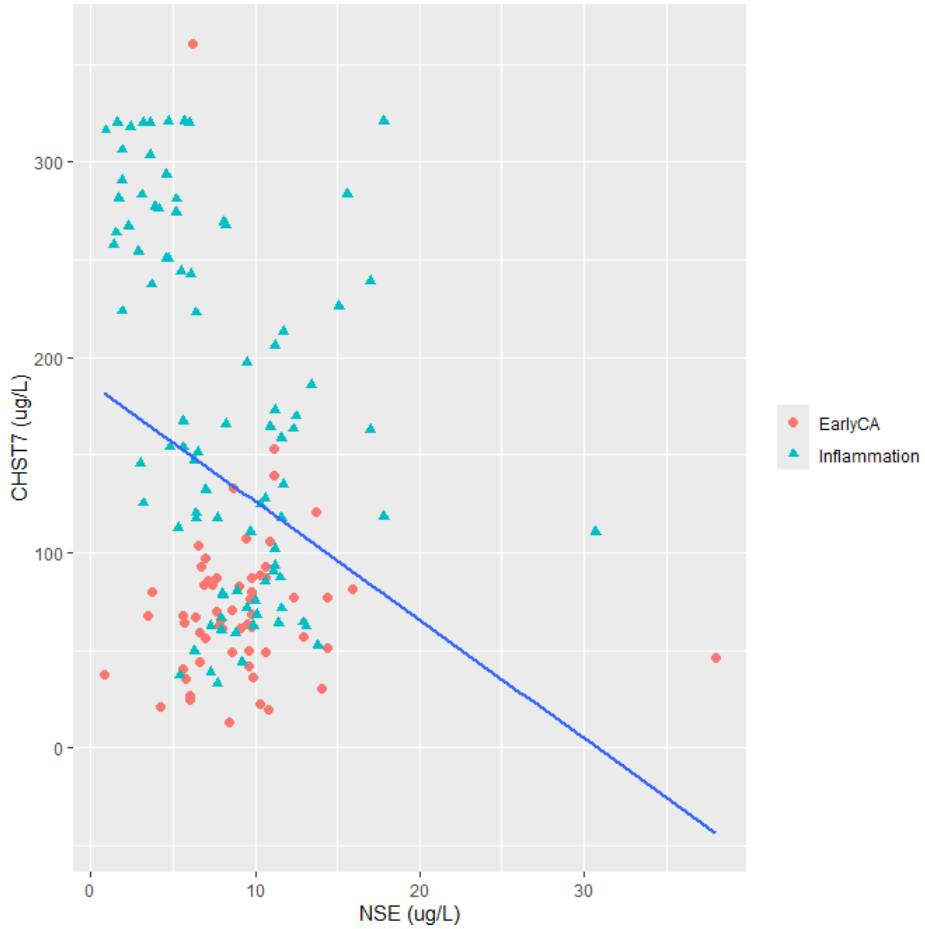

Figure 10. Correlation between Carbohydrate sulfotransferase 7 (CHST7) and neuron specific enolase (NSE) (N = 147). Pearson correlation was used to assess the strength and direction of the linear relationship between variables, while linear regression was applied to model and quantify the relationship.

Pearson correlation analysis was also conducted to evaluate the relationship between serum CHST7 (µg/L) and CRP (mg/L) concentrations (Figure 11): r = 0.662 indicates a strong positive relationship between serum CHST7 and CRP concentrations ($P < 0.001$). This suggests that higher CRP concentrations were generally associated withhigher CHST7 concentrations.

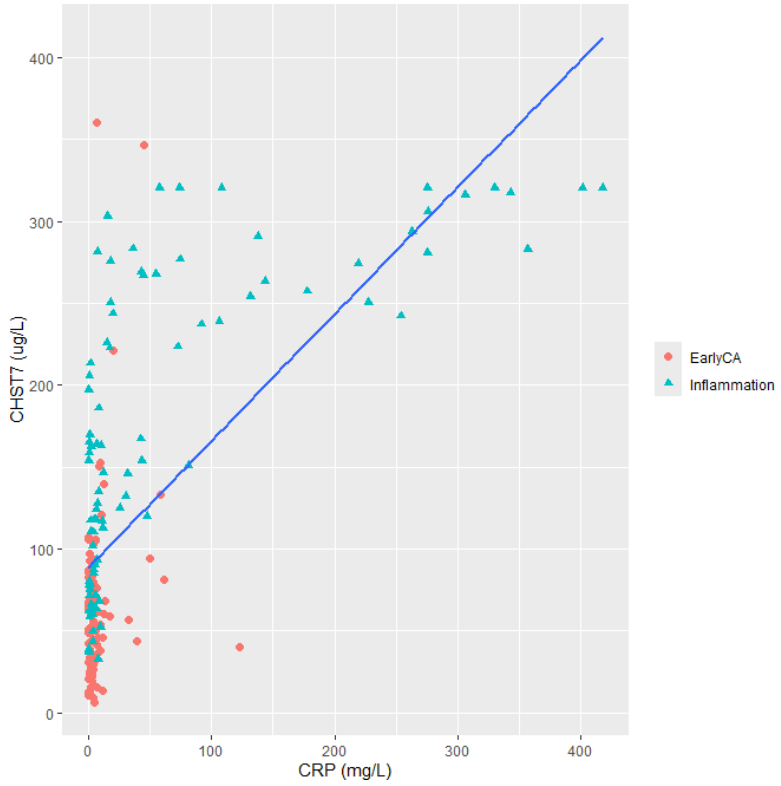

Figure 11. Correlation between carbohydrate sulfotransferase (CHST) and c reactive protein (CRP) (N = 197). Pearson correlation was used to assess the strength and direction of the linear relationship between variables, while linear regression was applied to model and quantify the relationship.

The scatter plot in Figure 12, represents the relationship between serum CHST7 and CRP in early-stage lung cancer patients with CRP > 5 mg/L. The analysis yielded a Pearson correlation coefficient of 0.070. The *P*-value of (*P* = 0.699) indicates that the observed correlation is not statistically significant. This implies that, in case of coexisting inflammation and early lung cancer, the positive correlation between CRP and proGRP is disrupted.

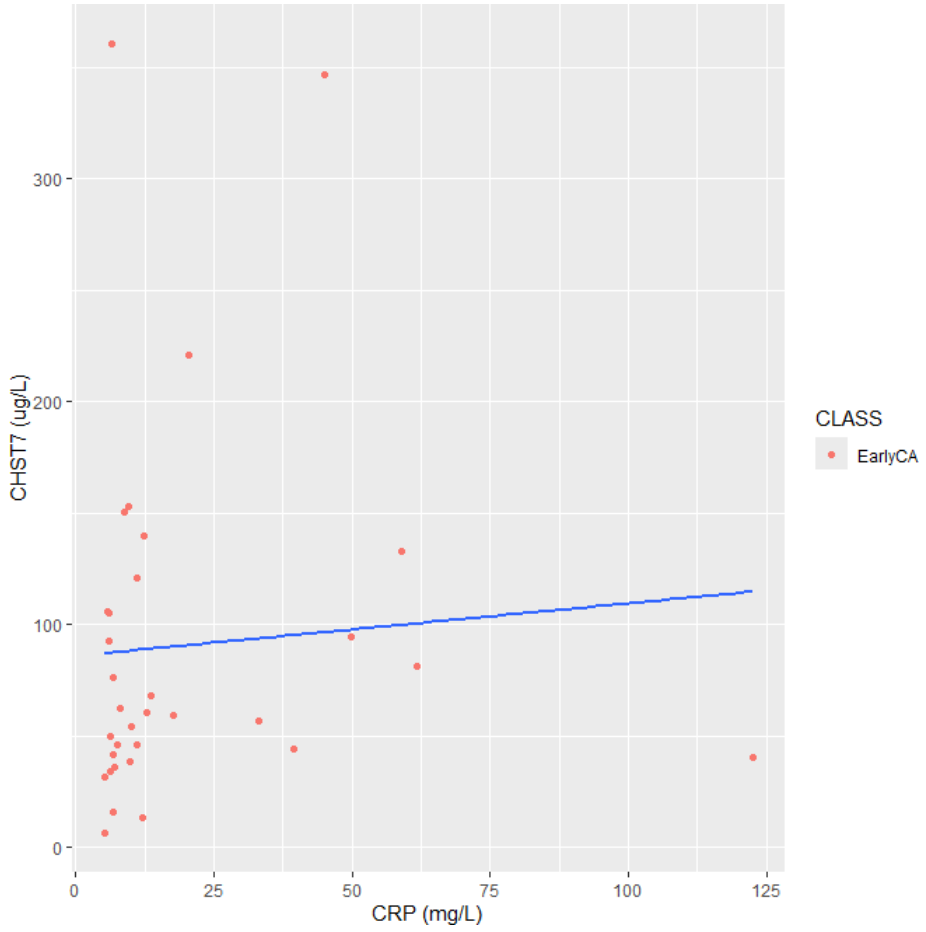

Figure 12. Relationship between carbohydrate sulfotransferase 7 (CHST7) and c reactive protein (CRP) in early lung cancer patients having CRP > 5 mg/L. Pearson correlation was used to assess the strength and direction of the linear relationship between variables, while linear regression was applied to model and quantify the relationship.

The scatter plot shown in Figure 13 illustrates the relationship between serum CHST7 and proGRP concentrations, providing a visual representation of the data distribution. The Pearson correlation coefficient of r = 0.158 suggests a weak positive linear relationship between the two variables, indicating that as serum CHST7 concentrations increase, proGRP concentrations also tend to increase, albeit to a small degree. Despite the weak correlation, the relationship is statistically significant, as evidenced by the p-value of $P = 0.026$.

The weak positive correlation indicates that while there is a measurable association between CHST7 and proGRP concentrations, it is not particularly strong. However, this modest relationship may still hold clinical relevance, as it implies that CHST7 and proGRP could provide complementary information when considered together, particularly in the context of early-stage lung cancer and lung inflammation. By reflecting different biological pathways or mechanisms, these biomarkers may offer a more comprehensive understanding of disease processes.

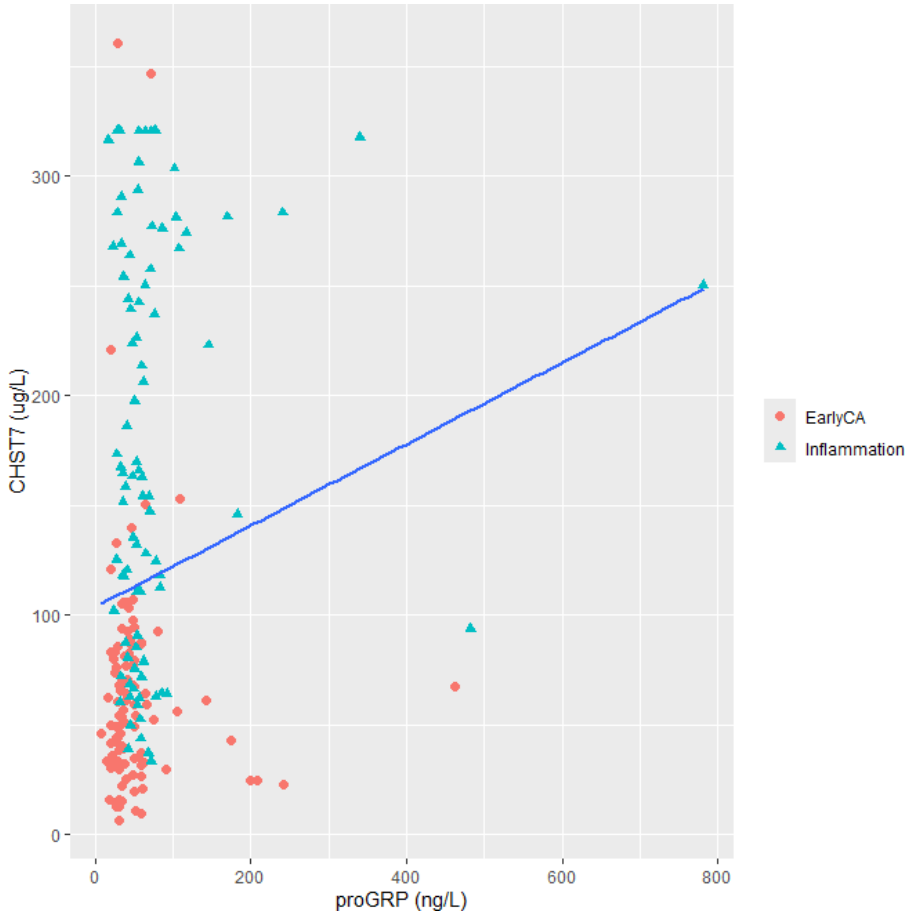

Figure 13. Relationship between serum carbohydrate sulfotransferase (CHST7) and pro gastrin releasing peptide (proGRP) concentrations. Pearson correlation was used to assess the strength and direction of the linear relationship between variables, while linear regression was applied to model and quantify the relationship.

5.5. CEA analysis

Figure 14 displays Box-and-Whisker plots that illustrate the distribution of serum CEA concentrations in individuals with early-stage lung cancer and those with non-malignant pulmonary inflammations. The high *P*-value of 0.957 suggests that there was no significant difference in serum CEA concentrations between individuals with early-stage lung cancer and those with non-malignant pulmonary inflammations. This indicates that CEA is not auseful marker for distinction between these two conditions.

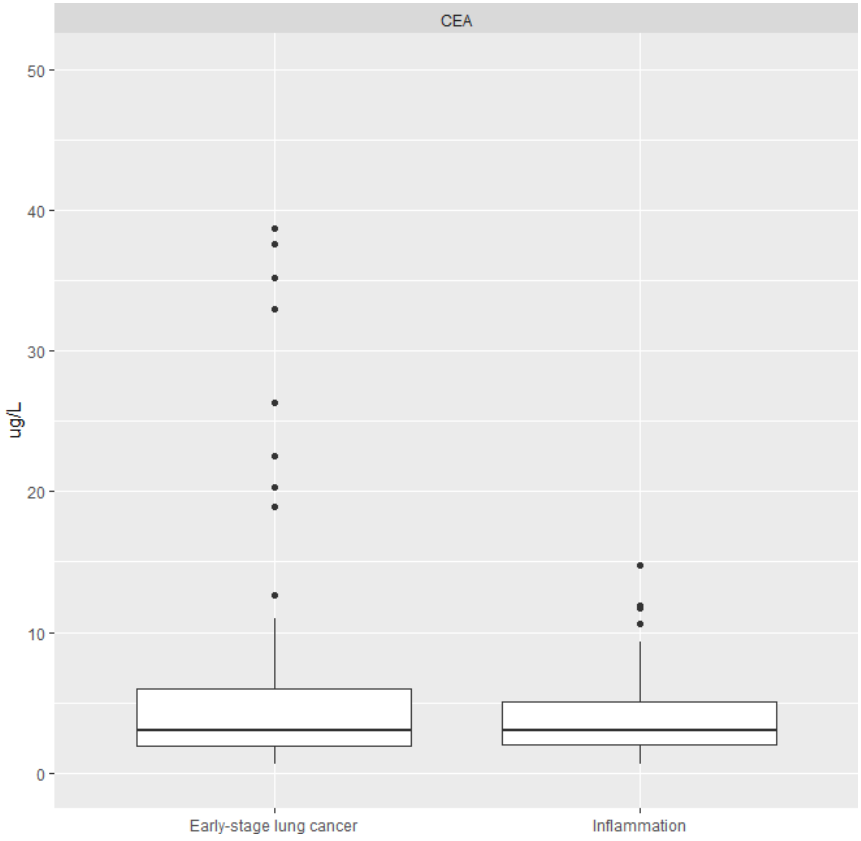

Figure 14. Box and Whisker plots. Serum carcinoembryonic antigen (CEA) in early lung cancer and non-malignant lung inflammations. X axis represent pathological states while Y axis represent CEA concentration. The statistical analysis using the Mann-Whitney U test provides insight into the difference between the two groups.

5.6. CYFRA 21-1 analysis

Box and Whisker plots showing serum CYFRA 21-1 distributions in lung cancer and non-malignant pulmonary inflammations are given in Figure 15. The Mann-Whitney U test for CYFRA 21-1 concentrations between the two groups produced a *P*-value of 0.390, indicating that there was no statistically significant difference in serum CYFRA21-1 concentrations between individuals with early-stage lung cancer and those with pulmonary inflammations.

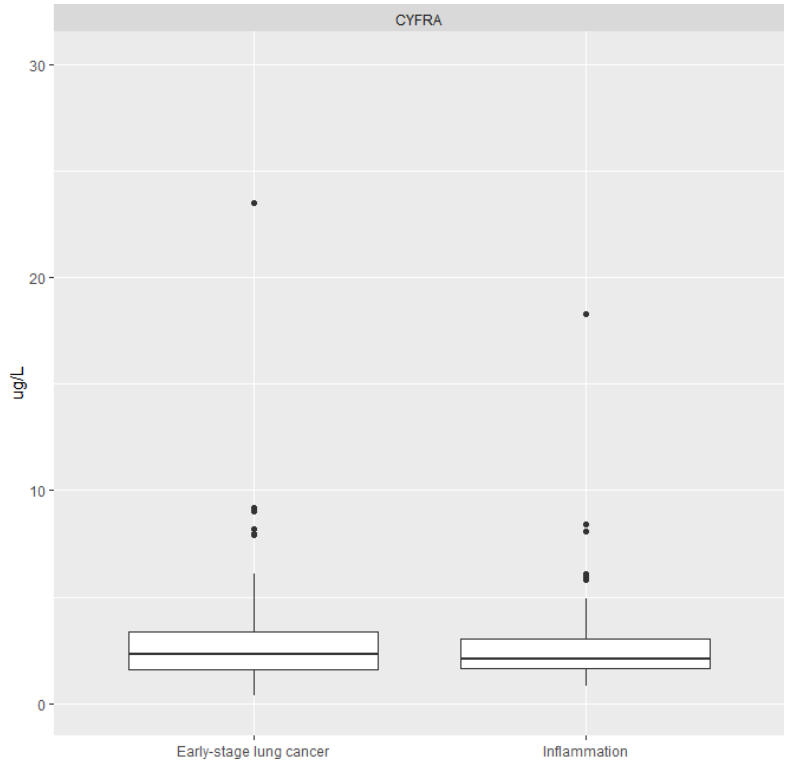

Figure 15. Box and Whisker plots of cytokeratin 19 fragment (CYFRA21-1) in early lung cancer and non-malignant lunginflammations. X axis represent pathological states while Y axis represent CYFRA 21-1 concentration. The statistical analysis using the Mann-Whitney U test provides insight into the difference between the two groups.

5.7. ROC ANALYSIS

Diagnostic accuracy of CEA, CYFRA, proGRP and CHST7 in differentiation between NSCLC and benign pulmonary inflammations was evaluated using ROC curve analysis (Figure 16). The ROC curve for CEA (AUC = 0.520) lies quite close to the diagonal line, indicating poor diagnostic performance. In CYFRA 21-1 case the ROC curve was also close to the diagonal. With an AUC of 0.541, CYFRA also exhibited no diagnostic capability. In contrast, the ROC curves for proGRP and CHST7 lie closer to the top left-hand corner of the graph, indicating significantly better diagnostic properties compared to CEA and CYFRA (Table 6). An AUC of 0.749 for proGRP and 0.864 for CHST7 reflects a moderateto good concertation of diagnostic accuracy. This suggests that proGRP and CHST7 have a substantial ability to differentiate between patients with early-stage NSCLC and those with benign inflammation.

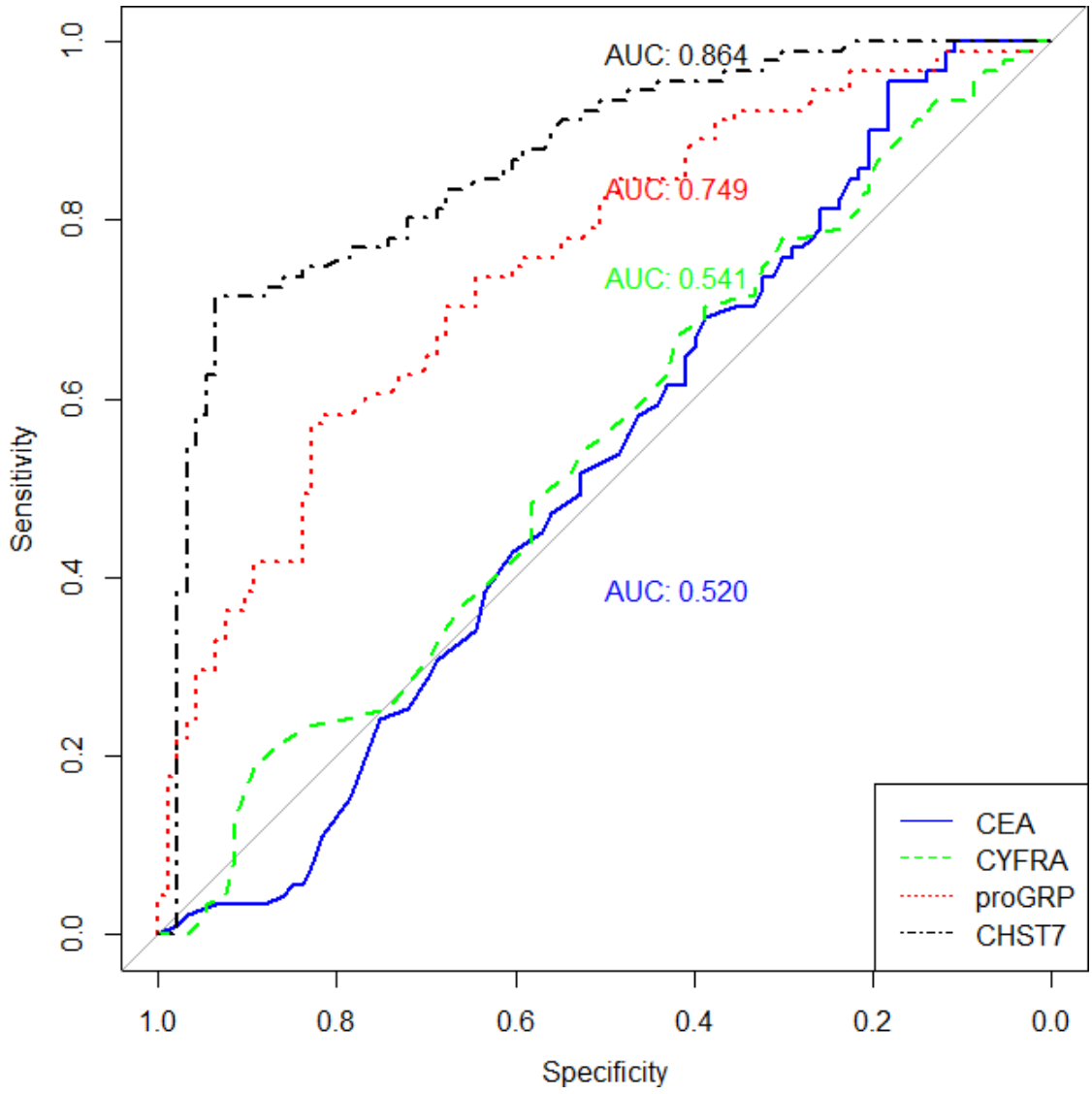

Figure 16. Differentiation between non-malignant lung inflammations and early-stages of Adenocarcinoma (ADC) and squamous cell carcinoma SQCC of lungs: ROC curve (N = 184). Receiver Operating Characteristic (ROC) curve analysis was conducted to evaluate the diagnostic accuracy of carcinoembryonic antigen (CEA), cytokeratin 19 fragment (CYFRA 21-1), pro gastrin releasing peptide (proGRP) and carbohydrate sulfotransferases 7 (CHST7) for detecting ADC and SQCC. De Long method was used for the area under the curve (AUC), sensitivity, and specificity at various cut-off points calculations.

Table 7. ROC analysis: differentiation between non-malignant lung inflammations and early stages of ADC and SQCC of lungs

Marker	**AUC**	***P***	**Cut Off**	**Sensitivity (%)**	**Specificity (%)**
CEA (µg/L)	0.520	0.641	9.3	18.3	95.6
CYFRA (µg/L)	0.541	0.341	2.7	38.7	70.3
proGRP (ng/L)	*0.749*	*<0,001*	51.8	81.7	58.2
CHST7 (µg/L)	*0.864*	*<0,001*	107.1	93.5	71.4

Receiver Operating Characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of four biomarkers: carcinoembryonic antigen (CEA), cytokeratin 19 fragment (CYFRA 21-1), pro gastrin releasing peptide (proGRP), and Carbohydrate sulfotransferase (CHST7). The area under the curve (AUC), sensitivity, specificity, and optimal cut-off values were determined for each marker. Statistical significance was assessed with *P*-values, where $P < 0.050$ indicates a significant result.

6. DISCUSSION

The primary objective of this study was to enhance the diagnostic accuracy in differentiating early-stage lung cancer from lung inflammations by identifying potential underlying etiologies. The specific aims of the study were to evaluate the diagnostic significance of novel biomarkers CHST7 and proGRP in distinguishing between NSCLC and benign inflammatory conditions. Additionally, the research sought to compare the diagnostic effectiveness of these markers with established tumor markers, such as CEA, CYFRA21-1, and NSE, to assess their relative sensitivity and specificity. By investigating these markers in conjunction with clinical data, the study aimed to determine their potential in improving early detection, providing more accurate differentiation between malignancy and benign diseases, and ultimately guiding clinical decision-making in the management of patients presenting with suspected lung conditions. This research holds promise for advancing diagnostic methods, enhancing early intervention, and reducing misdiagnoses in lung cancer detection.

Our findings are consistent with those reported by Begolli et al., who demonstrated that proGRP concentrations may be associated with early-stage ADC or SQCC (78). Conversely, higher proGRP concentrations, in addition to their established association with NETs, were found to correlate with acute inflammatory conditions, such as pneumonia or COPD-E. Notably, in COPD-E, proGRP levels may even overlap with those observed in SCLC, further complicating differentiation in certain cases. Among the markers evaluated, proGRP was shown to significantly enhance diagnostic models for distinguishing early-stage ADC, SQCC, and NET from pneumonia and COPD (78).

The findings of this study also indicate that proGRP and, more notably, CHST7, show a significant relationship with CRP, a well-established marker of inflammation. This association suggests that both proGRP and CHST7 may function as acute-phase reactants, playing a role in the body's inflammatory response. Although proGRP is primarily recognized as a biomarker for NETs and SCLC, the observed strong correlation with CRP raises the possibility that proGRP may also be involved in the acute inflammatory response, independent of malignancy. This dual role of proGRP implies that elevated concentrations of this marker could not only reflect tumor activity, as traditionally understood, but also be influenced by inflammatory conditions, complicating its diagnostic interpretation. Such findings emphasize the need for careful consideration of proGRP levels in clinical practice, as they may not solely be indicative of cancer but could also be a response to inflammation, potentially affecting the accuracy of its use in distinguishing between malignancy and inflammatory diseases. Further research is necessary to clarify the specific mechanisms underlying this relationship and to determine how proGRP and CHST7 can be utilized most effectively in clinical diagnostics.

The strong association of CHST7 with CRP points to its likely role as an acute- phase reactant. CHST7 may be upregulated during inflammation, contributing to or responding to inflammatory processes. This is particularly noteworthy as CHST7's involvement in inflammation could suggest a broader biological function, potentially linking it to both immune response and cancer progression.

The analysis reveals that proGRP does not correlate with NSE, despite both beingused biomarkers for the diagnosis of NET and SCLC. The absence of a correlation between proGRP and NSE implies that these two biomarkers are likely influenced by distinct biological processes or pathways within NET and SCLC. While both are elevatedin these

malignancies, the triggers or conditions that lead to their increased expression may differ. This divergence in expression pathways could reflect the complex and heterogeneous nature of neuroendocrine tumors, where different subsets of cells or molecular pathways are responsible for the production of proGRP and NSE. This findingemphasizes the importance of using both markers in a complementary manner for a morenuanced understanding of these states.

When comparing COPD-R and COPD-E, it is evident that the concentrations of proGRP and CHST7 are lower and more consistent during remission. However, these concentrations increase and become more unpredictable during exacerbations. The increased concertation during exacerbations could signal an environment conducive to tumor development or progression, making it a potential biomarker for both COPD severity and associated tumorigenesis. By adopting this integrated approach, there is the potential for improved COPD treatment and an ensuing reduction in the incidence of related comorbidities such as lung cancer.

The distribution of proGRP status presented through the Box and Whisker plots indicatedthe patients with SCLC had the highest concentration of proGRP and the patients with NSCLC had the lowest concentration of proGRP. The ROC analysis proved the ability ofthe marker to differentiate NSCLC at an early stage. Furthermore, the Mann-Whitney U test provided additional evidence to support the diagnostic potential of proGRP, which yielded a *P*-value of <0.001 showing the significant difference in proGRP concentrations between the two groups. The statistical significance of the identified differences shows the potential of proGRP as a discriminative marker. The fact that proGRP can differentiate between malignant and benign pulmonary diseases strengthens its practical application in clinical practice.

Some interesting relations were observed when linear regression analysis was performed to compare proGRP with NSE and CRP. There is a weak negative correlationbetween proGRP and NSE: this could mean that these markers function differently in lung cancer. On the contrary, there was a moderately positive correlation between proGRP and CRP: it suggests that proGRP is related to inflammation, which is frequentlyobserved in malignant tumors. In certain contexts, proGRP and CHST7 provided valuable information, particularly when inflammation is a part of a malignant disease etiology, despite not being primary markers for inflammation like CRP or cytokines (e.g., IL-6, TNF-α). In complex cases that involve both cancer and inflammatory processes, the expectedcorrelations between CRP, CHST7, and proGRP are disrupted, suggesting that these biomarkers provide distinct information under these conditions. Unlike the typical patternobserved in uncomplicated inflammation where CHST7 and proGRP concentrations rise,the simultaneous occurrence of a tumor alters this dynamic. This indicates that the tumorexerts a significant influence on the inflammatory processes, thereby modifying the usualbiomarker response patterns.

The ROC analysis for proGRP showed an AUC of 0.749 and 81.7% sensitivity and58.2% specificity. This performance also suggests that proGRP is relatively sensitive in differentiating NSCLC from inflammations, though its specificity rating is relatively low. Therefore, combining proGRP with other diagnostic methods for diagnosing NSCLC agrees with the study objectives in increasing the efficiency of early NSCLC diagnosis. Specifically, dissecting CHST7, Box and Whisker plots showed that medianconcentrations decreased, and variability was reduced in NSCLC patients relative to non-malignant inflammation patients. This differential expression provides evidence that CHST7 can be involved in inflammation and may be used for initial diagnostic evaluation. Correlation analysis of CHST7, NSE and CRP showed a significant negative correlation with NSE and positive

correlation with CRP. These correlations indicate that CHST7 is associated with inflammation and, indirectly, it may be associated with tumor metabolic reprogramming.

High AUC denotes that CHST7 has a very high ability to differentiate early-stage lung cancer from other benign inflammations involving the lungs. Conclusively, the high capacity of CHST7 for diagnosis strongly suggests the introduction of CHST7 could increase the reliability of diagnoses and aid in treatment of patients. However, it is important to interpret the results cautiously due to the significant variations observed in the control samples. Regardless, the result should be interpreted in combination with current established biomarkers.

In this study, it was noted that the diagnostic efficiency of CEA and CYFRA 21-1 was rather low in comparison to that observed which conflicts with the literature findings (105 - 107): however, it should be kept in mind that, in this study, only the early-stage of NSCLC cases and inflammations were analyzed. Box and Whisker plots for these markers indicated an insignificant difference between the group of NSCLC patients and the non-malignant cases in terms of interquartile ranges. ROC analysis provided nearly zero discriminating ability and showed an AUC close to 0.5. These markers are not

sensitive or specific for diagnosing early-stage NSCLC. Such findings call for better diagnostic tests for these conditions.

Limitations observed in CEA and CYFRA 21-1 cases augment the need for more specific markers. On the other hand, proGRP and CHST7 have the potential to enhancediagnostic accuracy and patient treatment as both markers offer unique and specific diagnostic information. Moreover, the study indicates that proGRP is elevated in the earlystages of certain tumors, particularly SCLC, and this elevation is independent of inflammatory processes. Meanwhile, its weak correlation with CHST7 suggests that bothbiomarkers could

be effectively used together to monitor disease activity and potential tumor development in COPD patients.

Given the primary aim of the current study to improve diagnostic sensitivity for early-stage pulmonary cancers, the existing literature on the heterogeneity and underlying causes of pulmonary malignancies strongly aligns with the objectives of this research. Accurate diagnosis of lung cancer often requires the use of multiple biomarkers, as the disease presents with various characteristics that can overlap with other pulmonary conditions (108-109). Notably, the diagnostic potential of biomarkers like CHST7 and proGRP has been highlighted in recent studies, especially in relation to early detection of non-small cell lung cancer (NSCLC) (61). Detecting early signs of lung cancer is particularly challenging, as symptoms in the early stages are often subtle or absent, which leads to a high proportion of patients being diagnosed at a more advanced or terminal stage (110). The biomarkers being evaluated in this study, including proGRP and CHST7, have shown promising potential in addressing this diagnostic gap, offering a more reliable means of early detection. Their use could significantly contribute to improved outcomes by identifying the disease before it reaches an advanced stage, thus enabling earlier intervention and potentially improving survival rates. This study underscores the importance of refining diagnostic tools and strategies for early-stage lung cancer detection to overcome the challenges posed by the asymptomatic nature of the early disease.

The literature review reveals that biomarkers like CEA, CYFRA21-1, and NSE have been primarily explored in the context of prognostic and predictive assessments for lung cancer (45, 62, 63, 85). These markers have shown utility in predicting the progression and treatment response of lung cancer, but their diagnostic accuracy, especially in the early

stages of the disease, remains a topic of concern. In line with previous studies, our findings suggest that these biomarkers exhibit limited diagnostic value when it comes to distinguishing early-stage malignancies from benign pulmonary conditions, such as infections and inflammatory diseases.

This limitation underscores a key challenge in clinical practice—relying on traditional markers to differentiate between malignant and non-malignant conditions, particularly in the early stages of lung cancer. At this stage, lung cancer is often asymptomatic or presents with symptoms that overlap with those of common inflammatory diseases, which makes it difficult to arrive at a definitive diagnosis. The low sensitivity and specificity of these markers in early-stage disease can lead to either false-negative results, delaying treatment, or false-positive results, leading to unnecessary interventions. This issue emphasizes the importance of developing and validating more reliable biomarkers that can offer improved diagnostic accuracy for early-stage lung cancer. Our study contributes to this ongoing effort by highlighting the potential of novel biomarkers, such as CHST7 and proGRP, which may offer better diagnostic differentiation and improve the clinical management of patients with suspected lung cancer.

One notable finding of this study is the distinct behavior of proGRP. While proGRP levels were observed to increase in inflammatory conditions, they remained effective in aiding the exclusion of NSCLC. This highlights its potential as a valuable diagnostic marker. Similarly, Kim et al. demonstrated that plasma proGRP concentration is highly sensitive and specific for distinguishing SCLC from nonmalignant conditions and NSCLC (111).

Our findings further support these conclusions by emphasizing proGRP's diagnostic utility, in the presence of inflammatory conditions. However, caution is necessary, as SCLC can also produce elevated proGRP levels, which may overlap with values seen in inflammatory conditions. This highlights the need for careful interpretation of proGRP concentrations, particularly in cases where SCLC cannot be ruled out, to ensure accurate differential diagnosis and minimize potential diagnostic uncertainties.

This overlap between elevated proGRP levels in inflammatory conditions and malignancies underscores the complexity of interpreting proGRP concentrations in clinical practice. While proGRP has been identified as a valuable marker for distinguishing SCLC from other lung conditions, its utility in distinguishing NSCLC from benign inflammatory diseases is more nuanced. As demonstrated in previous studies, including Dumesny et al. (112), the synthesis of proGRP can be upregulated in response to inflammation within the pulmonary system, which is often characterized by the activation of immune cells such as macrophages and neutrophils. These immune cells, upon activation, release pro-inflammatory cytokines and chemokines that can contribute to the production of proGRP in the lungs (73).

These findings suggest that inflammation within the lung can lead to elevated proGRP levels, which complicates its use as a definitive diagnostic marker for malignancy. The pro-inflammatory cytokines associated with inflammation are not only involved in the body's immune response but are also implicated in the pathophysiology of lung cancer, creating a potential confounding factor when proGRP is used to diagnose early-stage NSCLC. This highlights the dual role that inflammation may play in both promoting carcinogenesis through sustained immune responses and complicating the interpretation of diagnostic markers. While proGRP may effectively distinguish between SCLC and other cancers, its elevation in

inflammatory conditions warrants caution when used for differentiating between NSCLC and benign diseases.

Furthermore, it has been proposed that SCLC cells can produce proGRP in response to pro-inflammatory signaling molecules, blurring the distinction between malignant and inflammatory conditions. This finding emphasizes the need for careful interpretation of proGRP results in clinical diagnostics, as the overlap in biomarker levels between inflammation and malignancy could lead to diagnostic errors. To enhance accuracy, proGRP should ideally be evaluated in conjunction with other biomarkers or clinical parameters to account for its variability in response to inflammation and malignancy. Overall, this study reinforces the importance of understanding the interplay between inflammation, biomarker expression, and lung cancer progression to improve diagnostic strategies.

The findings of this study highlight the diagnostic potential of CHST7, a promising biomarker candidate, which demonstrated significantly higher concentrations in non-malignant lung inflammations. This supports its application in differentiating inflammatory conditions from malignant ones. CHST7, known for its role in cellular signaling and structural activities, has diagnostic and prognostic value as evidenced by previous literature (113). The observed differences in CHST7 levels between malignant and non-malignant conditions further confirm its involvement in inflammatory processes and its utility in distinguishing early-stage lung cancer from inflammation. These findings align with earlier reports on the role of CHST7 in differentiating NSCLC from inflammation (88, 113).

The presence and function of tumor-infiltrating lymphocytes (TILs) are also critical to the development and prognosis of NSCLC, particularly in smokers with COPD, who are at

elevated risk (109, 110). This underscores the need for comprehensive analysis of the interactions between signaling pathways and biomarkers, such as CHST7, in their contribution to both cancer and inflammation. Such insights enhance the theoretical understanding of biomarker research and provide a foundation for developing innovative diagnostic approaches. These approaches could facilitate earlier cancer detection, optimize treatment selection, and improve patient outcomes.

The diagnostic application of biomarkers like proGRP and CHST7 suggests a shift toward a multi-biomarker approach, which could significantly improve the detection rate of NSCLC, particularly in its early stages. This approach could be integrated into routine screening protocols for high-risk groups, enabling timely and accurate diagnoses. Early detection through screening is critical for improving survival rates and enhancing quality of life, making it a socially significant tool in managing lung cancer (11). Incorporating these biomarkers into standard diagnostic procedures holds promise for increased precision in both diagnosis and treatment, potentially reducing the overall burden of lung cancer on healthcare systems.

Shabana et al. demonstrated that CRP elevation in NSCLC is closely associated with tumor size and disease staging (114). These findings align with the well-documented role of systemic inflammation in tumor progression, as CRP levels often reflect the presence and extent of an inflammatory response elicited by the tumor. When comparing these results with the findings of the current study, some similarities and distinctions emerge.

In our study, CRP was included as one of the markers analyzed in the differentiation of NSCLC from inflammatory conditions. Although the reduced sample size for CRP data, posed some limitations and our findings suggest that CRP does play a role in identifying

inflammatory processes associated with malignancy. However, our data did not specifically examine the relationship between CRP levels and tumor size or stage. Instead, the focus was on evaluating CRP in the context of its utility alongside other biomarkers, such as CHST7 and proGRP, for diagnostic purposes.

Interestingly, while Shabana et al. highlighted CRP's correlation with tumor burden, our findings underscore the importance of considering CRP in combination with other markers to enhance diagnostic specificity and sensitivity. CRP, as a non-specific inflammatory marker, may rise not only due to malignant processes but also in response to benign pulmonary conditions such as pneumonia or chronic obstructive pulmonary disease exacerbations (114). However, when integrated with markers like proGRP, which demonstrated superior diagnostic accuracy in this study, CRP could provide complementary information that aids in distinguishing malignancy from benign inflammation.

The differing contexts and focuses of the two studies highlight the multifaceted nature of CRP's diagnostic potential. While Shabana et al. emphasized its prognostic value in relation to tumor size and stage, our study illustrates its role within a diagnostic framework, particularly when evaluated alongside other biomarkers (114). Future research should aim to combine these perspectives, exploring the interplay between CRP levels, tumor characteristics, and its integration into multi-marker diagnostic models for a more comprehensive understanding of its utility in NSCLC management.

Furthermore, the correlation analysis reinforces the importance of understanding biomarkers in disease processes. The link between CRP, proGRP, and CHST7 in inflammation appears to be indirect, with CHST7 likely playing a more prominent role in the inflammatory

environment. Pulmonary inflammation may elevate CRP levels and pro-inflammatory cytokines, potentially altering the glycosylation of surface proteins on cancer cells through changes in the expression of enzymes like CHST7 (115). These mechanisms could provide critical insights into the interplay between inflammation and cancer, further supporting the diagnostic utility of CHST7 in separating inflammatory conditions from early-stage lung malignancies.

Finally, the ROC analysis of CHST7 in this study revealed an AUC value of 0.864, which highlights its strong diagnostic capability and is consistent with findings reported in the literature (64). This high AUC value underscores the potential of CHST7 as a reliable biomarker for distinguishing lung cancer from benign pulmonary inflammations. Such diagnostic accuracy is particularly valuable in clinical settings, where early and accurate differentiation between malignant and non-malignant conditions remains a critical challenge (116).

The promising diagnostic performance of CHST7 suggests that it could play a significant role in improving diagnostic algorithms for lung cancer, especially when used alongside other established or novel biomarkers. By providing a clearer and more reliable distinction between malignancy and inflammation, CHST7 could contribute to reducing diagnostic uncertainty, minimizing the risk of misdiagnosis, and avoiding unnecessary invasive procedures that can be both costly and burdensome for patients. Moreover, this could ultimately support the timely initiation of appropriate treatments, which is crucial for improving patient outcomes in lung cancer cases. The use of CHST7 could thus enhance the precision and efficiency of current diagnostic approaches, offering a more comprehensive tool for clinicians. However, while these findings are encouraging, there remains a need for further investigation into

CHST7's diagnostic properties. Additional research is needed, particularly in the context of other pulmonary diseases, to assess its sensitivity, specificity, and overall utility in clinical practice. This would ensure that CHST7 can be effectively integrated into broader diagnostic strategies, enhancing its value and potential in the early detection and management of lung cancer.

Future research should explore the potential of CHST7 in differentiating not only lung cancer from inflammation but also from other pulmonary conditions such as pulmonary fibrosis, sarcoidosis, and interstitial lung diseases. These conditions often present with overlapping clinical, radiological, and biochemical features, which can significantly complicate the diagnostic process and increase the risk of misdiagnosis. The ability to accurately distinguish between these conditions and lung cancer is essential for developing targeted therapeutic strategies and ensuring optimal patient care. Expanding the scope of CHST7 research could provide valuable insights into its broader utility and help establish it as a versatile and reliable biomarker in pulmonary medicine. Such an advancement could have significant implications for improving diagnostic accuracy and reducing the burden on patients and healthcare systems alike. Additionally, studies with larger, more diverse patient populations, including different age groups, ethnicities, and disease stages, are essential to validate the generalizability of these findings. This would allow for a more comprehensive understanding of CHST7's performance and reliability across various clinical scenarios and disease contexts. By examining its diagnostic capabilities in a wide range of pulmonary conditions, researchers can assess its potential role in routine clinical practice. Such efforts would further strengthen the case for incorporating CHST7 into routine diagnostic practices and pave the way for more effective management of pulmonary diseases, ultimately improving

patient outcomes.

Overall, the current study complements the theoretical model set in the body of literature that discusses the application of multiple biomarkers in the screening and early detection of lung cancer. Specifically, the results referring to proGRP and CHST7 provide compelling evidence that the employment of various markers for the early detection of NSCLC significantly increases diagnostic accuracy. This enhanced diagnostic capability is expected to contribute to more favorable clinical outcomes for patients, enabling earlier and more precise identification of the disease. Early detection, in turn, can facilitate timely intervention and more tailored therapeutic strategies, which are crucial for improving survival rates and quality of life. Thus, the outcomes of the study strongly support the significance of these biomarkers in the diagnostic process and establish a solid foundation for further research and development. The study's findings open avenues for exploring the broader application of these biomarkers in clinical practice, potentially leading to improved screening protocols and more personalized treatment options. Additionally, the results pave the way for future investigations into how the combination of multiple biomarkers can be used to optimize diagnostic algorithms, enhance therapeutic decision-making, and ultimately contribute to better patient outcomes. As such, the study highlights the growing potential of proGRP, CHST7, and other biomarkers in advancing the precision medicine approach in lung cancer management.

The present study's findings provide valuable theoretical contributions to the diagnostic strategies for early-stage pulmonary malignancies, offering new insights into how multiple biomarkers can enhance clinical decision-making. By clinically investigating the performance of proGRP and introducing CHST7 as a novel candidate biomarker, this study not only expands upon existing knowledge but also strengthens the theoretical framework supporting the use of a multifaceted approach to disease differentiation. The integration of multiple biomarkers, such

as proGRP and CHST7, allows for a more comprehensive evaluation of patients, increasing the likelihood of accurate early-stage diagnosis, particularly in distinguishing pulmonary malignancies from other benign or inflammatory conditions. This study, by exploring the unique diagnostic potential of these markers, contributes to a deeper understanding of their role in improving early detection and differential diagnosis, which is critical for successful treatment and management of pulmonary diseases. In doing so, the research underscores the growing importance of multi-marker strategies in clinical practice and advances the scientific foundation necessary for their future application in real-world diagnostic settings. (117, 118). This approach is particularly relevant in addressing the critical challenges associated with the early and accurate diagnosis of malignant pulmonary diseases, where the overlap of clinical and biochemical profiles between malignancies and inflammatory conditions often complicates the diagnostic process. The incorporation of proGRP and CHST7 into diagnostic models demonstrates the potential to refine current methods by enhancing their specificity and sensitivity, paving the way for more precise differentiation between malignant and benign pulmonary conditions. Furthermore, these findings highlight the importance of integrating novel biomarkers alongside established markers, such as CEA, CYFRA21-1, and NSE, to develop a more robust diagnostic toolkit. In doing so, the study not only strengthens the theoretical underpinnings of multi-biomarker strategies but also provides a foundation for future research aimed at optimizing early diagnostic approaches for pulmonary malignancies, ultimately improving clinical outcomes.

The combined use of CHST7 and proGRP holds significant promise for providing valuable insights into both the inflammatory processes and potential carcinogenesis in patients with COPD. By incorporating CHST7 and proGRP into routine diagnostic and monitoring protocols, clinicians could achieve a more nuanced understanding of disease progression,

distinguishing between benign inflammatory conditions and the early stages of cancer development. This integration has the potential to enhance diagnostic accuracy, particularly in identifying subtle changes indicative of malignancy at its earliest and most treatable stages. Moreover, these biomarkers could help in stratifying patients based on their risk profiles, enabling tailored surveillance programs and more personalized treatment strategies. Improved diagnostic performance through the use of CHST7 and proGRP could ultimately lead to earlier detection, timely interventions, and better overall outcomes for COPD patients at risk of developing lung cancer. This approach underscores the importance of biomarker-driven diagnostics in bridging the gap between chronic inflammation and carcinogenesis, offering a significant step forward in the management of this high-risk population. The introduction and verification of CHST7 as a diagnostic marker for differentiation of NSCLC from inflammation is the most important theoretical contribution.It is confirmed that CHSTs are associated with cellular signaling and structural activities in pulmonary inflammation, confirming their potential diagnostic and prognostic use (119). International research on the prevalence of lung cancer and mortality statistics identifies crucial changes while stressing the significance of early detection and therapy (22). Though there has been progress in the field of diagnostics, delays still occur and hence there is a need to enhance ways of early involvement. Some of the proposed changes tostaging include improving the prognosis's reliability and treatment outcomes. The findingsof the study provide an innovative approach as compared to the existing methods that suggest low CHST7 concentrations in NSCLC patients as compared to those with benigninflammations making it a good candidate for differentiation from inflammations. CHST7 does not follow the rise of CRP in malignant diseases associated with inflammations whatimproves CHST7 diagnostic reliability. This agrees with the theoretical understanding thatCHSTs including

CHST7 are involved in the differentiation between cancer-related and inflammatory events (16, 93). The highlighted relationships between CHST7, NSE, and CRP that were found in the study imply that these markers are involved in multifaceted signaling regarding cancer progression and inflammation. This finding is in concordance with the discussed theory that markers could shed light on the biological mechanisms ofcancer inception and progression, thus improving the diagnostic and prognostic potential(120-122). The study results highlight the need to consider other molecular markers for diagnosing NSCLC. This theoretical implication arouses the possibilities of subsequent research on CHST7 and other markers' pathways with the ultimate goal of enhancing thechances of early diagnosis and patient prognosis.

Promising diagnostic performances of proGRP and CHST7 offers possibilities in differentiating between malignant and benign pulmonary diseases (72). This classificationis important in clinical scenarios because it defines the next course of action. For instance, combining proGRP with other biomarkers such as CEA and NSE can help betterdiagnose SCLC and NSCLC cases: it can prevent misdiagnosis which may result in the wrong treatment being administered to the patient. Using a biomarker panel of proGRP and CHST7 offers an improved method of increasing diagnostic certainty and minimizing invasive procedures like biopsies which have adverse effects on the patient's health. Thispractical application can be linked to the study's goal of enhancing diagnostic sensitivity and specificity to help clinicians possess efficient methods for diagnosing NSCLC at an early stage.

The study also focuses on the necessity of integrating new biomarkers into the available frameworks of healthcare systems. Currently assessing the expression and activity of CHST7 may be useful in clinical applications, particularly in comprehending its involvement

in different disorders, such as cancer and inflammatory conditions. Currently available methods may not provide the necessary sensitivity or specificity to detect accurate concentration of CHST7 or distinguish it from other comparable enzymes. More reliable analytical techniques are needed that have the potential to improve the reliabilityof CHST7 assessment, especially when dealing with complex biological samples.

Finally, the possibility of future development of marker investigation with the addition of various markers into clinical practice is pointed out. This discovery of CHST7as a suitable marker implies the central message of the continued effort and expansion of the research and innovations in the oncology field. It can also follow this continual studyto examine the molecular processes through which markers are synthesized and their function in cancer development.

In conclusion, this study provides valuable insights into the potential of proGRP and CHST7 biomarkers for differentiating NSCLC from inflammatory conditions, particularly in early diagnostic contexts. The findings suggest that these biomarkers could play a crucial role in improving the accuracy of diagnoses and facilitating the early detection of lung cancer. As highlighted in our study, proGRP demonstrated a strong ability to distinguish between SCLC and NSCLC, though its application may be complicated by elevated levels in inflammatory conditions. CHST7, on the other hand, showed promise in discriminating between malignant and benign pulmonary diseases, further supporting its potential as a complementary tool in clinical diagnostics.

Moving forward, the integration of proGRP and CHST7 into clinical management will require several steps to optimize their utility. The transition from research settings to routine clinical use will depend on increased standardization of biomarker testing methods to ensure

consistent and reliable results across various healthcare environments. Additionally, the training of healthcare professionals in the correct interpretation and application of these biomarkers is essential to maximize their diagnostic value. Given the complexity of lung cancer and its differentiation from other pulmonary diseases, future studies should focus on refining the diagnostic algorithms that incorporate these markers, potentially exploring their synergistic use with other established or novel biomarkers to enhance diagnostic sensitivity and specificity.

Moreover, the findings of this study underscore the need for ongoing research into additional biomarkers that could further improve the early detection and treatment of lung cancer. The dynamic nature of cancer progression and its overlapping symptoms with benign inflammatory conditions necessitate the development of a more comprehensive biomarker panel that can better differentiate between malignant and non-malignant diseases. By pursuing these avenues of research, we can continue to enhance the efficiency of cancer diagnosis, leading to more personalized and effective treatment strategies for patients with lung cancer. Ultimately, this study calls for a broader commitment to refining diagnostic methods and expanding the repertoire of biomarkers available for clinical use, with the goal of improving patient outcomes.

6.1. Limitations

The following limitations should be taken into consideration: firstly, 198 participants were enrolled in the study as a sample group, but the focus laid on individuals from Zagreb University Clinical center determines limitations about a population. This has restricted the applicability of the results to other population of distinct characteristics. Also, the study results were mostly exploratory, pointing out associations rather than causality. These limitations justify

continued research to replicate the study using a sample of a wider population, and more people of different demographics, to support the conclusion.

In this study, the availability of diagnostic markers NSE and CRP was somewhat restricted, with NSE data being available for only 149 subjects and CRP data for 197 subjects out of the total cohort of 198 individuals. This reduced sample size for these specific markers represents a notable limitation, as it restricts the study's ability to comprehensively evaluate their diagnostic utility across the entire population. Such constraints may have impacted the robustness of the conclusions drawn regarding their roles in differentiating between malignant and inflammatory pulmonary conditions. Nevertheless, the analysis conducted with the available data suggests that both NSE and CRP contribute valuable insights when used alongside CHST7 and proGRP, particularly in understanding the interplay between malignancy and inflammation.

Additionally, the control group utilized in this study comprised exclusively cases of pulmonary inflammation, without including other non-malignant lung conditions such as pulmonary fibrosis, benign lung nodules, or interstitial lung diseases. This lack of representation from a broader spectrum of non-malignant conditions presents another limitation, as it narrows the scope of the study and may reduce the generalizability of the findings to other clinical scenarios. The absence of data on these additional conditions means that the ability of CHST7, proGRP, NSE, and CRP to differentiate lung cancer from a more diverse range of non-malignant lung diseases could not be fully assessed.

This limitation is particularly critical in clinical practice, where patients frequently present with various pulmonary conditions that may exhibit overlapping features with malignancy.

Including a more heterogeneous control group in future studies would offer a more nuanced and comprehensive evaluation of the diagnostic utility of these biomarkers, better representing the real-world clinical challenges faced by healthcare professionals. Expanding the study population to encompass a broader range of non-malignant lung diseases, including infectious, autoimmune, and chronic inflammatory conditions, would also help validate the specificity and sensitivity of the markers. Such expansion would increase the robustness of these biomarkers and facilitate their potential integration into clinical workflows, where precise differentiation between lung cancer and other pathologies is essential for effective management.

Despite these limitations, the findings of this study underscore the potential of CHST7 and proGRP as promising biomarkers in distinguishing lung cancer from inflammatory conditions. Furthermore, the complementary roles of NSE and CRP in conjunction with these biomarkers highlight the growing recognition of multi-marker strategies to improve diagnostic accuracy. By combining multiple biomarkers, clinicians can enhance diagnostic precision, reduce the risk of misdiagnosis, and tailor treatment strategies more effectively. However, larger-scale studies with more diverse patient cohorts and comprehensive control groups are needed to substantiate these findings. Such studies will be crucial in confirming the biomarkers' reliability and enhancing their applicability across a wider spectrum of pulmonary diseases. With continued research, the integration of these biomarkers into clinical practice could offer a more accurate, timely, and individualized approach to patient care.

7. CONCLUSION AND RECOMMENDATIONS

7.1. Summary of Results

This study had the objective of the diagnostic accuracy improvement in differentiation between early-stage pulmonary cancer and benign pulmonary inflammations. Thus, the main goals of this work were as follows: 1) assessing the clinical relevance of novel marker CHST7 and 2) comparing its diagnostic properties to other well-known tumor markers, including CEA, CYFRA21-1, NSE, and pointedly proGRP. The results showed that CHST7 is a promising marker for differentiation of early lung cancer from lung inflammations. Besides, concentrations of proGRP were demonstrated to outperform other routinely used markers for accurately differentiation of malignant conditions from benign pulmonary inflammations.

proGRP demonstrated a good differential diagnosis between SCLC and NSCLC, as can also be seen in our findings. However, according to our findings, proGRP also differentiates between NSCLC stage 1 and 2 and inflammations, with the latter having highervalues in comparison with the NSCLC group of patients, which can be a good indicator of the differentiation between these two conditions.

7.2. Recommendations

Considering the presented results, the following recommendations for further research and clinical interventions are suggested. Firstly, subsequent investigations are required to demonstrate the diagnostic accuracy of CHST7 in various patient groups and contexts. These studies should be centered on the objective of confirming the biomarker's efficiency in the different lung cancer stages and interacting with other biomarkers. It would be beneficial to determine how proGRP and CHST7 interact with other diagnostic markers in more detail to fine-tune its use and enhance the diagnostic process.

Secondly, it is suggested that future works should focus on exploring the molecular mechanism of CHST7 and its relationship with inflammation, cancer growth and metastasis. To the lesser extent, the same is true for the proGRP. Development of more reliable assays and protocols for the measurement of CHST7 may improve NSCLC diagnostics. Further research should aim at creating reliable and objective testing models that may be integrated into currently existing approaches to screening and diagnostics. It is also imperative that healthcare professionals be trained in how to properly interpret and apply the concentration of proGRP and CHST7.

Lastly, more research can be done on how proGRP and CHST7 can be incorporated with other advanced diagnostic methods. This is expected to provide a more comprehensive and precise diagnostics of lung cancer at an early stage. Interdisciplinary efforts are needed to transition these developments towards the clinic for the benefit of the patients.

8. ABSTRACT

Objectives: The study aimed to improve diagnostic accuracy in differentiating early-stage lung cancer from benign pulmonary inflammations. Key objectives included assessing the clinical relevance of the novel biomarker CHST7 and comparing it with established markers such as CEA, CYFRA21-1, NSE, and proGRP. It also sought to evaluate the diagnostic utility of CHST7 and proGRP in distinguishing NSCLC from inflammation and COPD.

Study Design: A clinical case-control study was conducted from 2020 to 2022 atClinical Hospital Centre Zagreb and Osijek University Hospital Centre, Croatia. The study enrolled 198 participants divided into two groups: those with early-stage lung cancer (stage I or II) and those with non-malignant pulmonary conditions (COPD and pneumonia).

Materials and Methods: CHST7 levels were measured using ELISA, while CEA,CYFRA 21-1, NSE and proGRP were analyzed via ECLIA method. All assays followed standardized protocols with internal and external quality controls.. Statistical tests, including the Mann-Whitney U and ROC analyses, assessed each marker's ability to differentiate lung cancer from inflammatory conditions.

Results: CHST7 and proGRP significantly improved diagnostic accuracy in distinguishing early-stage lung cancer from benign pulmonary inflammations. CHST7 levels were highest in pneumonia (263.1 µg/L) and COPD (131.0 µg/L), compared to 73.4 µg/L in early-stage lung cancer. ROC analysis showed CHST7 had an AUC of 0.864, with 93.5% sensitivity and 71.4% specificity at a 107.1 µg/L cutoff, outperforming traditional markers.

proGRP also effectively differentiated early-stage NSCLC from inflammation, with an AUC of 0.749, sensitivity of 81.7%, and specificity of 58.2%. Median proGRP levels were elevated in inflammation, particularly in pneumonia (94.9 ng/L), compared to early-stage lung cancer (51.8 ng/L). Traditional markers like CEA and CYFRA 21-1 had AUCs near 0.5, indicating limited diagnostic value in early stages.

Conclusion: CHST7 and proGRP demonstrated strong diagnostic potential in distinguishing early-stage lung cancer from inflammatory conditions. proGRP effectively differentiated SCLC from NSCLC and distinguished early-stage NSCLC from inflammation, with inflammation cases showing higher proGRP values. CHST7 exhibited high sensitivity and specificity, making it a promising biomarker for early lung cancer detection. Integrating CHST7 and proGRP into diagnostic workflows could significantly enhance early lung cancer differentiation from benign pulmonary inflammations.

Keywords: Early stage lung cancer, CHST7, proGRP, Diagnostic accuracy, Biomarker

9. SAŽETAK

Ciljevi istraživanja: Studija je imala za cilj poboljšati dijagnostičku točnost u razlikovanju ranog stadija raka pluća od benignih plućnih upala. Ključni ciljevi uključivali su procjenu kliničke relevantnosti novog biomarkera CHST7 i njegovu usporedbu s etabliranim markerima poput CEA, CYFRA21-1, NSE i proGRP. Također je ispitana dijagnostička korisnost CHST7 i proGRP u razlikovanju NSCLC-a od upale i KOPB-a.

Dizajn studije: Klinička studija slučaj-kontrola provedena je od 2020. do 2022. u KBC Zagreb i KBC-u Osijek, Hrvatska. Studija je obuhvatila 198 sudionika podijeljenihu dvije skupine: u ranim stadijem raka pluća (stadij I ili II) i oni s nemalignim plućnim stanjima (KOPB i upala pluća).

Materijali i metode: Razine CHST7 određene su ELISA metodom, dok su CEA, CYFRA 21-1, NSE i proGRP analizirani metodom ECLIA. Svi testovi provedeni su prema standardiziranim protokolima s internim i eksternim kontrolama kvalitete. Statističke analize, uključujući Mann-Whitney U test i ROC analize, procijenile su sposobnost svakog markera u razlikovanju raka pluća od upalnih stanja.

Rezultati: CHST7 i proGRP značajno su poboljšali dijagnostičku točnost u razlikovanju ranog stadija raka pluća od benignih plućnih upala. Razine CHST7 bile su najviše u upali pluća (263,1 µg/L) i KOPB-u (131,0 µg/L), u usporedbi s 73,4 µg/L kod ranog stadija raka pluća. ROC analiza pokazala je da CHST7 ima AUC od 0,864, s osjetljivošću od 93,5% i specifičnošću od 71,4% pri graničnoj vrijednosti od 107,1 µg/L, nadmašujući tradicionalne markere. proGRP je također učinkovito razlikovao rani stadij NSCLC-a od upale, s AUC-

om od 0,749, osjetljivošću od 81,7% i specifičnošću od 58,2%. Medijan razine proGRP bio je povišen kod upalnih stanja, osobito kod upale pluća (94,9 ng/L), u usporedbi s ranim stadijem raka pluća (51,8 ng/L). Tradicionalni markeri poput CEA i CYFRA 21-1 imali su AUC blizu 0,5, što ukazuje na njihovu ograničenu dijagnostičku vrijednost u ranim stadijima.

Zaključak: CHST7 i proGRP pokazali su visok dijagnostički potencijal u razlikovanju ranog raka pluća od upalnih stanja. proGRP je učinkovito razlikovao SCLC od NSCLC-a, dok je CHST7 pokazao visoku osjetljivost i specifičnost, čineći ga obećavajućim biomarkerom. Njihova integracija u dijagnostičke protokole može poboljšati ranu detekciju raka pluća..

Ključne riječi: rani stadij raka pluća, CHST7, proGRP, dijagnostička točnost, biomarker

10. REFERENCES

1. Moldoveanu B, et al. Inflammatory mechanisms in the lung. J Inflamm Res. 2008;1–11.
2. Schwartz AG, Cote ML. Epidemiology of lung cancer. Lung Cancer Pers Med Curr Knowl Ther. 2016;21–41.
3. Şahin F, Aslan AF. Relationship between inflammatory and biological markers and lung cancer. J Clin Med. 2018 Jun 25;7(7):160. doi: 10.3390/jcm7070160. PMID: 29941786; PMCID: PMC6069225.
4. Larsson K. Aspects on pathophysiological mechanisms in COPD. J Intern Med. 2007;262(3):311–340.
5. Barnes PJ. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol. 2016;138(1):16–27.
6. Parris BA, O'Farrell HE, Fong KM, Yang IA. Chronic obstructive pulmonary disease (COPD) and lung cancer: common pathways for pathogenesis. J Thorac Dis. 2019;11(Suppl 17):S2155.
7. Valavanidis A, Vlachogianni T, Fiotakis K, Loridas S. Pulmonary oxidative stress, inflammation, and cancer: respirable particulate matter, fibrous dusts, and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms. Int J Environ Res Public Health. 2013;10(9):3886–3907.
8. El-Shimy WS, El-Dib AS, Nagy HM, Sabry W. A study of IL-6, IL-8, and TNF-α as inflammatory markers in COPD patients. Egypt J Bronchol. 2014;8:91–99.
9. Di Rosanna P, Salvatore C. Reactive oxygen species, inflammation, and lung diseases. Curr Pharm Des. 2012;18(26):3889–3900.
10. Azad N, Rojanasakul Y, Vallyathan V. Inflammation and lung cancer: roles of reactive

oxygen/nitrogen species. J Toxicol Environ Heal Part B. 2008;11(1):1–15.

11. Aaron SD. Management and prevention of exacerbations of COPD. BMJ. 2014;349.
12. Berry CE, Wise RA. Mortality in COPD: causes, risk factors, and prevention. COPD J Chronic Obstr Pulm Dis. 2010;7(5):375–382.
13. Porter J. Detection of early lung cancer. Thorax. 2000;55(Suppl 1):S56.
14. Inage T, Nakajima T, Yoshino I, Yasufuku K. Early lung cancer detection. Clin Chest Med. 2018;39(1):45–55.
15. Blandin Knight S, Crosbie PA, Balata H, Chudziak J, Hussell T, Dive C. Progress and prospects of early detection in lung cancer. Open Biol. 2017;7(9):170070.
16. Kalinke L, Thakrar R, Janes SM. The promises and challenges of early non-small cell lung cancer detection: patient perceptions, low-dose CT screening, bronchoscopy, and biomarkers. Mol Oncol. 2021;15(10):2544–2564.
17. Nicholson AG, et al. The 2021 WHO classification of lung tumors: impact of advances since 2015. J Thorac Oncol. 2022;17(3):362–387.
18. Thomas A, Liu SV, Subramaniam DS, Giaccone G. Refining the treatment of NSCLC according to histological and molecular subtypes. Nat Rev Clin Oncol. 2015;12(9):511–526.
19. Drilon A, Rekhtman N, Ladanyi M, Paik P. Squamous-cell carcinomas of the lung: emerging biology, controversies, and the promise of targeted therapy. Lancet Oncol. 2012;13(10):e418–e426.
20. Derman BA, Mileham KF, Bonomi PD, Batus M, Fidler MJ. Treatment of advanced squamous cell carcinoma of the lung: a review. Transl Lung Cancer Res. 2015;4(5):524.
21. H. Hoy, T. Lynch, and M. Beck, “Surgical treatment of lung cancer,” Crit. Care Nurs. Clin., vol. 31, no. 3, pp. 303–313, 2019.

22. S. M. Gadgeel, S. S. Ramalingam, and G. P. Kalemkerian, “Treatment of lung cancer,” Radiol. Clin., vol. 50, no. 5, pp. 961–974, 2012.
23. Durham AL, Adcock IM. The relationship between COPD and lung cancer. Lung Cancer. 2015 Nov;90(2):121-7. doi:10.1016/j.lungcan.2015.08.017. Epub 2015 Aug 29. PMID: 26363803; PMCID: PMC4718929.
24. Parris BA, O'Farrell HE, Fong KM, Yang IA. Chronic obstructive pulmonary disease (COPD) and lung cancer: common pathways for pathogenesis. J Thorac Dis. 2019 Oct;11(Suppl 17):S2155-S2172. doi: 10.21037/jtd.2019.1.54. PMID: 31737343; PMCID: PMC6831920.
25. Gould MK, Donington J, Lynch WR, Mazzone PJ, Midthun DE, Naidich DP, Wiener RS. Evaluation of individuals with pulmonary nodules: when is it lung cancer? Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest. 2013 May;143(5 Suppl):e93S-e120S. doi: 10.1378/chest.12-2351. PMID: 23649456; PMCID: PMC3749714.
26. Nooreldeen, R.; Bach, H. Current and Future Development in Lung Cancer Diagnosis. Int. J. Mol. Sci. 2021, 22, 8661. https://doi.org/10.3390/ijms22168661.
27. Silva, M., Picozzi, G., Sverzellati, N. et al. Low-dose CT for lung cancer screening: position paper from the Italian college of thoracic radiology. Radiol med 127, 543–559 (2022). https://doi.org/10.1007/s11547-022-01471-y.
28. Kapeleris, J., Ebrahimi Warkiani, M., Kulasinghe, A., Vela, I., Kenny, L., Ladwa, R & Punyadeera, C. Clinical applications of circulating tumor cells and circulating tumor DNA in non-small cell lung cancer—an update. Frontiers in oncology, 12, 859152. https://doi.org/10.3389/fonc.2022.859152.
29. Gobbini, E., Swalduz, A., Giaj Levra, M., Ortiz-Cuaran, S., Toffart, A. C., Perol, M., &

Saintigny, P. Implementing ctDNA analysis in the clinic: challenges and opportunities in non-small cell lung cancer. Cancers, 12(11), 3112. https://doi.org/10.3390/cancers12113112.

30. Stansel, T., Wickline, S. A., & Pan, H. NF-κB inhibition suppresses experimental melanoma lung metastasis. Journal of cancer science and clinical therapeutics, 4(3), 256. doi: 10.26502/jcsct.5079070.
31. Locke, W. J., Guanzon, D., Ma, C., Liew, Y. J., Duesing, K. R., Fung, K. Y., & Ross, J. P. DNA methylation cancer biomarkers: translation to the clinic. Frontiers in genetics, 10, 1150. https://doi.org/10.3389/fgene.2019.01150.
32. De Chiara, L., Leiro-Fernandez, V., Rodríguez-Girondo, M., Valverde, D., Botana-Rial, M. I., & Fernández-Villar, A. Comparison of bisulfite pyrosequencing and methylation-specific qPCR for methylation assessment. International Journal of Molecular Sciences, 21(23), 9242. https://doi.org/10.3390/ijms21239242.
33. Liang, R., Li, X., Li, W., Zhu, X., & Li, C. DNA methylation in lung cancer patients: Opening a 'window of life' under precision medicine. Biomedicine & Pharmacotherapy, 144, 112202. https://doi.org/10.1016/j.biopha.2021.112202.
34. Yu Z., Chen H., Ai J., Zhu Y., Li Y., Borgia J. A., Yang J., Zhang J., Jiang B., Gu W., Deng Y. Global lipidomics identified plasma lipids as novel biomarkers for early detection of lung cancer. Oncotarget. 2017; 8: 107899-107906. Retrieved from https://www.oncotarget.com/article/22391/.
35. Gandhi, Z.; Gurram, P.; Amgai, B.; Lekkala, S.P.; Lokhandwala, A.; Manne, S.; Mohammed, A.; Koshiya, H.; Dewaswala, N.; Desai, R.; et al. Artificial Intelligence and Lung Cancer: Impact on Improving Patient Outcomes. Cancers 2023, 15, 5236.
36. Kim, G., Park, C., Yoon, Y.K. et al. Prediction of lung cancer using novel biomarkers based

on microbiome profiling of bronchoalveolar lavage fluid. Sci Rep 14, 1691 (2024). https://doi.org/10.1038/s41598-024-52296-w.

37. G. Sutedja, “New techniques for early detection of lung cancer,” Eur. Respir. J., vol. 21, no. 39 suppl, pp. 57s-66s, 2003.
38. J. Vansteenkiste, C. Dooms, C. Mascaux, and K. Nackaerts, “Screening and early—detection of lung cancer,” Ann. Oncol., vol. 23, pp. x320–x327, 2012.
39. R. Nooreldeen and H. Bach, “Current and future development in lung cancer diagnosis,” Int. J. Mol. Sci., vol. 22, no. 16, p. 8661, 2021.
40. K. D. Hatzakis, M. E. Froudarakis, D. Bouros, N. Tzanakis, N. Karkavitsas, and N. M. Siafakas, “Prognostic value of serum tumor markers in patients with lung cancer,” Respiration, vol. 69, no. 1, pp. 25–29, 2002.
41. R. Molina *et al.*, “Assessment of a combined panel of six serum tumor markers for lung cancer,” *Am. J. Respir. Crit. Care Med.*, vol. 193, no. 4, pp. 427–437, 2016.
42. J. Schneider, “Tumor markers in detection of lung cancer,” *Adv. Clin. Chem.*, vol. 42, pp. 1–41, 2006.
43. H. Wu, Q. Wang, Q. Liu, Q. Zhang, Q. Huang, and Z. Yu, “The Serum Tumor Markers in Combination for Clinical Diagnosis of Lung Cancer.,” *Clin. Lab.*, vol. 66, no. 3, 2020.
44. D. Amicizia *et al.*, “Systematic review of lung cancer screening: advancements and strategiesfor implementation,” in *Healthcare*, MDPI, 2023, p. 2085.
45. G. Li *et al.*, “Serum markers CA125, CA153, and CEA along with inflammatory cytokines inthe early detection of lung cancer in high-risk populations,” *Biomed Res. Int.*, vol. 2022, 2022.
46. M. Nagpal, S. Singh, P. Singh, P. Chauhan, and M. A. Zaidi, “Tumor markers: A diagnostic tool,” *Natl. J. Maxillofac. Surg.*, vol. 7, no. 1, pp. 17–20, 2016.

47. C. Hao, G. Zhang, and L. Zhang, “Serum CEA levels in 49 different types of cancer and noncancer diseases,” *Prog. Mol. Biol. Transl. Sci.*, vol. 162, pp. 213–227, 2019

48. Campos-da-Paz, J. G. Dórea, A. S. Galdino, Z. G. M. Lacava, and M. de Fatima Menezes Almeida Santos, “Carcinoembryonic antigen (CEA) and hepatic metastasis in colorectal cancer: update on biomarker for clinical and biotechnological approaches,” *Recent Pat. Biotechnol.*, vol. 12, no. 4, pp. 269–279, 2018.

49. C. Hall *et al.*, “A review of the role of carcinoembryonic antigen in clinical practice,” *Ann. Coloproctol.*, vol. 35, no. 6, p. 294, 2019.

50. A. Fahim, M. G. Crooks, R. Wilmot, A. P. Campbell, A. H. Morice, and S. P. Hart, “Serum carcinoembryonic antigen correlates with severity of idiopathic pulmonary fibrosis,” *Respirology*, vol. 17, no. 8, pp. 1247–1252, 2012.

51. H. Dai *et al.*, “Increased lung cancer risk in patients with interstitial lung disease and elevatedCEA and CA 125 serum tumour markers,” *Respirology*, vol. 19, no. 5, pp. 707–713, 2014.

52. Takada, M, Masuda, N, Matsuura, E, Kusunoki, Y, Matui, K, Nakagawa, K, et al. “Measurement of cytokeratin 19 fragments as a marker of lung cancer by CYFRA 21-1 enzyme immunoassay”. Br J Canc 1995;71:160–5. https://doi.org/10.1016/0169-5002(95)98797-e.

53. Kulpa, J, Wójcik, E, Reinfuss, M, Kołodziejski, L. Carcinoembryonic antigen, squamous cellcarcinoma antigen, CYFRA 21-1, and neuron-specific enolase in squamous cell lung cancer patients. Clin Chem 2002;48:1931–7. https://doi.org/10.1093/clinchem/48.11.1931.

54. Molina, R, Auge, JM, Escudero, JM, Marrades, R, Viñolas, N, Carcereny, E, et al. Mucins CA 125, CA 19.9, CA 15.3 and TAG-72.3 as tumor markers in patients with lung cancer:

comparison with CYFRA 21-1, CEA, SCC and NSE. Tumour Biol 2008;29:371–80. https://doi.org/10.1159/000181180. Epub 2008 Dec 8.

55. A. S. Babkina, M. A. Lyubomudrov, M. A. Golubev, M. V Pisarev, and A. M. Golubev, “Neuron-Specific Enolase—What Are We Measuring?,” *Int. J. Mol. Sci.*, vol. 25, no. 9, p. 5040, 2024.

56. M. A. Isgrò, P. Bottoni, and R. Scatena, “Neuron-specific enolase as a biomarker: biochemicaland clinical aspects,” *Adv. cancer biomarkers from Biochem. to Clin. a Crit. Revis.*, pp. 125–143, 2015.

57. T. Shibayama *et al.*, “Complementary roles of pro-gastrin-releasing peptide (proGRP) and neuron specific enolase (NSE) in diagnosis and prognosis of small-cell lung cancer (SCLC),”*Lung cancer*, vol. 32, no. 1, pp. 61–69, 2001.

58. G.Kanakis and G.Kaltsas, “Biochemical markers for gastroenteropancreatic neuroendocrine tumours (GEP-NETs),” *Best Pract. Res. Clin. Gastroenterol.*, vol. 26, no. 6, pp. 791–802, 2012.

59. P. Capelli, M. Fassan, and A. Scarpa, “Pathology–grading and staging of GEP-NETs,” *Best Pract. Res. Clin. Gastroenterol.*, vol. 26, no. 6, pp. 705–717, 2012.

60. S. Mizuguchi *et al.*, “Serum Sialyl Lewisx and cytokeratin 19 fragment as predictive factors for recurrence in patients with stage I non-small cell lung cancer,” *Lung cancer*, vol. 58, no. 3, pp. 369–375, 2007.

61. S. Mizuguchi *et al.*, “Clinical value of serum cytokeratin 19 fragment and sialyl-Lewis x in non-small cell lung cancer,” *Ann. Thorac. Surg.*, vol. 83, no. 1, pp. 216–221, 2007.

62. M. Hassanein, J. C. Callison, C. Callaway-Lane, M. C. Aldrich, E. L. Grogan, and P. P. Massion, “The state of molecular biomarkers for the early detection of lung cancer,” *CancerPrev. Res.*, vol. 5, no. 8, pp. 992–1006, 2012.

63. I. Hoseok and J.-Y. Cho, “Lung cancer biomarkers,” *Adv. Clin. Chem.*, vol. 72, pp. 107–170,2015.

64. G. Yang, Z. Xiao, C. Tang, Y. Deng, H. Huang, and Z. He, “Recent advances in biosensor fordetection of lung cancer biomarkers,” *Biosens. Bioelectron.*, vol. 141, p. 111416, 2019.

65. R. Charkiewicz *et al.*, “Gene expression signature differentiates histology but not progressionstatus of early-stage NSCLC,” *Transl. Oncol.*, vol. 10, no. 3, pp. 450–458, 2017.

66. F. Chen, X.-Y. Wang, X.-H. Han, H. Wang, and J. Qi, “Diagnostic value of Cyfra21-1, SCCand CEA for differentiation of early-stage NSCLC from benign lung disease,” *Int. J. Clin. Exp. Med.*, vol. 8, no. 7, p. 11295, 2015.

67. Z. F. Jiang, M. Wang, and J. L. Xu, “Thymidine kinase 1 combined with CEA, CYFRA21-1and NSE improved its diagnostic value for lung cancer,” *Life Sci.*, vol. 194, pp. 1–6, 2018.

68. R. Molina *et al.*, “Usefulness of serum tumor markers, including progastrin-releasing peptide,in patients with lung cancer: correlation with histology,” *Tumor Biol.*, vol. 30, no. 3, pp. 121–129, 2009.

69. Li C, Ma QY, Liu XQ, Li HD et al. Genetic and pharmacological inhibition of GRPR protects against acute kidney injury via attenuating renal inflammation and necroptosis. Mol Ther. 2023 Sep 6;31(9):2734-2754. doi: 10.1016/j.ymthe.2023.06.016. Epub 2023 Jul 5. PMID: 37415332; PMCID: PMC10492025.

70. B. Nisman, H. Biran, N. Ramu, N. Heching, V. Barak, and T. Peretz, “The diagnostic and prognostic value of proGRP in lung cancer,” *Anticancer Res.*, vol. 29, no. 11, pp. 4827–4832,2009.

71. H.-J. Oh *et al.*, “Progastrin-releasing peptide as a diagnostic and therapeutic biomarker

of small cell lung cancer," *J. Thorac. Dis.*, vol. 8, no. 9, p. 2530, 2016.

72. E. Wojcik and J. K. Kulpa, "Pro-gastrin-releasing peptide (proGRP) as a biomarker in small-cell lung cancer diagnosis, monitoring and evaluation of treatment response," *Lung Cancer Targets Ther.*, pp. 231–240, 2017.

73. L. Chen *et al.*, "Inflammatory responses and inflammation-associated diseases in organs," *Oncotarget*, vol. 9, no. 6, p. 7204, 2018.

74. V. Barchiesi *et al.*, "Circulating progastrin-releasing peptide in the diagnosis of Small Cell Lung Cancer (SCLC) and in therapeutic monitoring,"*J.Circ.Biomarkers*,vol.10, p. 9, 2021.

75. R. Molina, X. Filella, and J. M. Augé, "proGRP: a new biomarker for small cell lung cancer,"*Clin. Biochem.*, vol. 37, no. 7, pp. 505–511, 2004.

76. Z. Gong *et al.*, "Overexpression of pro-gastrin releasing peptide promotes the cell proliferation and progression in small cell lung cancer," *Biochem. Biophys. Res. Commun.*, vol. 479, no. 2, pp. 312–318, 2016.

77. J. Dong *et al.*, "Progastrin-releasing peptide precursor and neuron-specific enolase predict theefficacy of first-line treatment with epidermal growth factor receptor (EGFR) tyrosine kinaseinhibitors among non-small-cell lung cancer patients harboring EGFR mutations," *Cancer Manag. Res.*, pp. 13607–13616, 2021.

78. Begolli G, Lukić M, Rumora L, Čorak L, Vukić Dugac A, Jakopović M, Samaržija M, Tomaš I, Knežević J, Debeljak Ž. "Serum progastrin-releasing peptide in pneumonia, chronic obstructive pulmonary disease and early-stage primary lung cancers". Biochem Med (Zagreb). 2025 Feb 15;35(1):010702. doi: 10.11613/BM.2025.010702. Epub 2024 Dec 15. PMID: 39703761; PMCID: PMC11654240.

79. W. Zhou, Y. Yang, Z. Wang, Y. Liu, and M. Lari Najafi, "Impact of HSP90α, CEA, NSE, SCC, and CYFRA21-1 on lung cancer patients," *J. Healthc. Eng.*, vol. 2021, 2021.

80. P. Paul, J. Suwan, J. Liu, J. S. Dordick, and R. J. Linhardt, “Recent advances in sulfotransferase enzyme activity assays,” *Anal. Bioanal. Chem.*, vol. 403, pp. 1491–1500, 2012.
81. J. R. Grunwell and C. R. Bertozzi, “Carbohydrate sulfotransferases of the GalNAc/Gal/GlcNAc6ST family,” *Biochemistry*, vol. 41, no. 44, pp. 13117–13126, 2002.
82. C. N. Falany, “Enzymology of human cytosolic sulfotransferases,” *FASEB J.*, vol. 11, no. 4,pp. 206–216, 1997.
83. K. G. Bowman and C. R. Bertozzi, “Carbohydrate sulfotransferases: mediators of extracellular communication,” *Chem. Biol.*, vol. 6, no. 1, pp. R9–R22, 1999.
84. C. E. Birse *et al.*, “Blood-based lung cancer biomarkers identified through proteomic discovery in cancer tissues, cell lines and conditioned medium,” *Clin. Proteomics*, vol. 12, pp. 1–12, 2015.
85. W.-M. Chang *et al.*, “The aberrant cancer metabolic gene carbohydrate sulfotransferase 11promotes non-small cell lung cancer cell metastasis via dysregulation of ceruloplasmin and intracellular iron balance,” *Transl. Oncol.*, vol. 25, p. 101508, 2022.
86. G. Begolli, I. Marković, J. Knežević, Ž. Debeljak. “Carbohydrate sulfotransferases: a review of emerging diagnostic and prognostic applications”. Biochem Med (Zagreb). 2023 Oct 15; 33(3):030503. doi: 10.11613/BM.2023.030503. Epub 2023 Aug 5. PMID: 37545696; PMCID: PMC10373059.
87. Y. Guan *et al.*, “Carbohydrate sulfotransferase 3 (CHST3) overexpression promotes cartilageendplate-derived stem cells (CESCs) to regulate molecular mechanisms related to repair of intervertebral disc degeneration by rat nucleus pulposus,” *J. Cell. Mol. Med.*, vol. 25, no. 13,pp. 6006–6017, 2021.
88. Ž. Debeljak *et al.*, “Serum carbohydrate sulfotransferase 7 in lung cancer and non-

malignantpulmonary inflammations," *Clin. Chem. Lab. Med.*, vol. 56, no. 8, pp. 1328–1335, 2018.

89. L. Zhang *et al.*, "Carbohydrate sulfotransferase 4 inhibits the progression of hepatitis B virus-related hepatocellular carcinoma and is a potential prognostic marker in several tumors," *Front. Oncol.*, vol. 10, p. 554331, 2020.
90. T. Zheng *et al.*, "Impairment of the autophagy-lysosomal pathway and activation of pyroptosis in macular corneal dystrophy," *Cell death Discov.*, vol. 6, no. 1, p. 85, 2020.
91. M. Tanaka-Okamoto, M. Mukai, H. Takahashi, Y. Fujiwara, M. Ohue, and Y. Miyamoto, "Various sulfated carbohydrate tumor marker candidates identified by focused glycomic analyses," *Glycobiology*, vol. 27, no. 5, pp. 400–415, 2017.
92. Y. Matsuda, Y. Fujii, M. Matsukawa, T. Ishiwata, M. Nishimura, and T. Arai, "Overexpression of carbohydrate sulfotransferase 15 in pancreatic cancer stroma is associatedwith worse prognosis," *Oncol. Lett.*, vol. 18, no. 4, pp. 4100–4105, 2019.
93. H. Zhou *et al.*, "CHST11/13 regulate the metastasis and chemosensitivity of human hepatocellular carcinoma cells via mitogen-activated protein kinase pathway," *Dig. Dis. Sci.*,vol. 61, pp. 1972–1985, 2016.
94. Q. Ren *et al.*, "Exploring the sulfate patterns of chondroitin sulfate/dermatan sulfate and keratan sulfate in human pancreatic cancer," *J. Pharm. Biomed. Anal.*, vol. 205, p. 114339, 2021.
95. H. Bi *et al.*, "CHST7 Gene methylation and sex-specific effects on colorectal cancer risk," *Dig. Dis. Sci.*, vol. 64, pp. 2158–2166, 2019.
96. F. C. Detterbeck and C. J. Gibson, "Turning gray: the natural history of lung cancer overtime," *J. Thorac. Oncol.*, vol. 3, no. 7, pp. 781–792, 2008.
97. National Institute for Health and Care Excellence: Guidelines (Internet). London:

National Institute for Health and Care Excellence (NICE); 2003-.https://www.ncbi.nlm.nih.gov/books/NBK11822/

98. Agustí A, Celli BR, Criner GJ, Halpin D, Anzueto A, et al.; Global Initiative for Chronic Obstructive Lung Disease 2023 Report: GOLD Executive Summary. Eur Respir J. 2023 Apr 1;61(4):2300239. doi: 10.1183/13993003.00239-2023. PMID: 36858443; PMCID: PMC10066569.

99. Travis WD, Brambilla E, Nicholson AG, Yatabe Y, Austin JHM, et al.; WHO Panel. The 2015 World Health Organization Classification of Lung Tumors: Impact of Genetic, Clinicaland Radiologic Advances Since the 2004 Classification. J Thorac Oncol. 2015 Sep;10(9):1243-1260. doi:10.1097/JTO 0000000000000630. PMID: 26291008.

100. Nehring SM, Goyal A, Patel BC. C Reactive Protein. [Updated 2023 Jul 10]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024-.Available from: https://www.ncbi.nlm.nih.gov/books/NBK441843/

101.Korse CM, Holdenrieder S, Zhi X, Zhang X, Qiu L, Geistanger A, Lisy MR, Wehnl B, van den Broek D, Escudero JM, Standop J, Hu M, Molina R. Multicenter evaluation of a new progastrin-releasing peptide (proGRP) immunoassay across Europe and China. Clin Chim Acta. 2015;438:388-95. doi: 10.1016/j.cca.2014.09.015.

102. Minamibata A, Kono Y, Arimoto T, Marunaka Y, Takayama K. Variability of serum CYFRA 21 - 1 and its susceptibility to clinical characteristics in individuals without cancer: a 4-year retrospective analysis. BMC Pulm Med. 2023 Sep 13; 23 (1):344. doi: 10.1186/s12890-023-02650-x. PMID: 37705035; PMCID: PMC10500899.

103. Kade Flowers. Neuron-Specific Enolase (NSE) analyte monograph. The Association

for Clinical Biochemistry and Laboratory Medicine. https://labmed.org.uk/static/a165f431-e3db-4467-b5b42396d64412a3/Neuron- Specific-Enolase-NSE-analyte-monograph.pdf. Date approved: 24 February 2023.

104. Kim D, Kwon H, Kim SM, Kim JS, Kim YJ, et al. (2024) Normal value of neuron-specific enolase for predicting good neurological outcomes in comatose out-of hospital cardiac arrestsurvivors. PLOS ONE 19(6): e0305771 https://doi.org/10.1371/journal.pone.0305771

105.T. Hanagiri et al., “Preoperative CYFRA 21-1 and CEA as prognostic factors in patients with stage I non-small cell lung cancer,” Lung cancer, vol. 74, no. 1, pp. 112–117, 2011.101.

106.K. Okamura, K. Takayama, M. Izumi, T. Harada, K. Furuyama, and Y. Nakanishi,“Diagnostic value of CEA and CYFRA 21-1 tumor markers in primary lung cancer,” Lung cancer, vol. 80, no. 1, pp. 45–49, 2013.

107. F. Barlési *et al.*, “Prognostic value of combination of Cyfra 21-1, CEA and NSE in patientswith advanced non-small cell lung cancer,” *Respir. Med.*, vol. 98, no. 4, pp. 357–362, 2004.

108. S. Ma *et al.*, “Multiplexed serum biomarkers for the detection of lung cancer,” *EBioMedicine*, vol. 11, pp. 210–218, 2016.

109. A. I. Robles and C. C. Harris, “Integration of multiple ‘OMIC’ biomarkers: A precision medicine strategy for lung cancer,” *Lung Cancer*, vol. 107, pp. 50–58, 2017.

110. P. Villalobos and I. I. Wistuba, “Lung cancer biomarkers,” *Hematol. Clin.*, vol. 31, no. 1, pp. 13–29, 2017.

111.Kim HR, Oh IJ, Shin MG, Park JS, Choi HJ, Ban HJ, Kim KS, Kim YC, Shin JH, Ryang

DW, Suh SP. Plasma proGRP concentration is sensitive and specific for discriminating small cell lung cancer from nonmalignant conditions or non-small cell lung cancer. J Korean Med Sci. 2011 May; 26(5):625-30. doi: 10.3346/jkms.2011.26.5.625. Epub 2011 Apr 21. PMID: 21532852; PMCID: PMC3082113.

112. Dumesny C, Patel O, Lachal S, Giraud AS, Baldwin GS, Shulkes A. Synthesis, expressionand biological activity of the prohormone for gastrin-releasing peptide (proGRP).Endocrinology. 2020 Jan 1; 147(1):52-9.4.

113. K. Ye and Y. Wang, “Long non-coding RNA ZNF674-AS1 antagonizes oxaliplatin resistance of gastric cancer via regulating EZH2-mediated methylation of CHST7,” *Aging (Albany NY)*, vol. 14, no. 13, p. 5523, 2022.

114. Shabana H, Refaat Sh. CRP evaluation in non-small cell lung cancer. Egyptian J Chest Dis Tuberc. 2014;63:10.1016/j.ejcdt.2014.02.003.

115. Hong S, Kang YA, Cho BC, Kim DJ. Elevated serum C-reactive protein as a prognostic marker in small cell lung cancer. Yonsei Med J. 2012 Jan;53(1):111-7. doi: 10.3349/ymj.2012.53.1.111. PMID: 22187240; PMCID: PMC3250314.

116.Lucchetta M, da Piedade I, Mounir M, Vabistsevits M, Terkelsen T, Papaleo E. Distinct signatures of lung cancer types: aberrant mucin O-glycosylation and compromised immune response. BMC Cancer. 2019;19:1-20.

117. Lin PY, Yu SL, Yang PC. MicroRNA in lung cancer. Br J Cancer. 2010;103(8):1144–1148.

118. Wu KL, Tsai YM, Lien CT, Kuo PL, Hung JY. The roles of MicroRNA in lung cancer. Int J Mol Sci. 2019;20(7):1611.

119. Yanaihara N, et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell. 2006;9(3):189–198.

120. Zamay N, et al. Current and prospective protein biomarkers of lung cancer. Cancers (Basel). 2017;9(11):155.

121. Seijo LM, et al. Biomarkers in lung cancer screening: achievements, promises, and challenges. J Thorac Oncol. 2019; 14(3):343–357.

122.Subramaniam S, Thakur RK, Yadav VK, Nanda R, Chowdhury S, Agrawal A. Lung cancer biomarkers: State of the art. J Carcinog. 2013 Feb 28;12:3. doi: 10.4103/1477-3163.107958. PMID: 23599685; PMCID: PMC3622361.

11. Curriculum Vitae

Name and Surname: Gramos Begolli

Address: Rr. Agim Cavderbasha nr.7, 10000 Prishtina, Kosovo

Telephone(s): +383 44 160 494

E-mail: gramosbegolli@gmail.com

Nationality: Kosovar

Date of Birth: 03.06.1980

Gender: Male

Education:

11/2011 – 10/2015	Specialist of Clinical Biochemistry – University Clinical Center
10/1998 – 07/2007	Doctor of Medicine, Medical Faculty – University of Prishtina
09/1994 – 06/1998	Gymnasium “Xhevdet Doda”, Pristina, Kosovo

Employment:

08/2017 – Present: Specialist of Clinical Biochemistry University Clinical Center of Kosovo – Medical Biochemistry Clinic, Pristina, Kosovo

10/2015 – Present: Lecturer “Diagnostic health science“, College “Heimerer”, Pristina, Kosovo

04/2016 - 02/2017: Specialist of Clinical Biochemistry, American Hospital, Pristina, Kosovo

02/2007 – 04/2016: Medical Doctor, “Bioticus” Medical Laboratory, Prishtina, Kosovo

Languages:

Mother tongue(s): Albanian

Other language(s): English (C1), Serbo-Croatian (C1), German (B1)

Membership:

Kosovo Medical Chamber

Kosovo Association of Clinical Chemistry

European Federation of Laboratory Medicine (EFLM)

Projects:

2002: Voluntary counseling and testing (VCT) to HIV / AIDS by PSI (Population Services International) and Kosovo Committee on HIV / AIDS, funded by USAID and "Save the Children"

2003: Training of trainers for voluntary counseling and testing of HIV / AIDS by PSI (Population Services International) and Kosovo Committee on HIV / AIDS, funded by USAID and "Save the Children".

2004: Training for the implementation of the required standards for the health care and quality Management techniques by the Ministry of Health of Kosovo.

Exchange Programs:

2023: IFCC PEP Program Institute of Laboratory Diagnostic Osijek Croatia,

2006: SCORA - Exchange program Sweden, 05.09 – 04. 10.2006

Seminars and Symposiums Participations:

2016: AAF Seminar „Lipid Metabolism“, Salzburg, Austria – Excellent Presentation Award

2015: 3rd EFLM-BD European Conference on Preanalytical Phase – Porto,Portugal

2012: EFCC Symposium "Education in Clinical Chemistry and Laboratory MEDICINE" in Prague, Czech Republic

2012: "New trends in classification, diagnosis and management of gastrointestinal disorders," EFCC Continuous Postgraduate Course in Clinical Chemistry Dubrovnik, Croatia

2009: Seminar in "monitoring and evaluating of National AIDS Program" of the WHO Collaborating Centre for Capacity Building in HIV Surveillance, Cavtat – Croatia

Congresses Abstract Presentations:

1. **Begolli G.** Correlation of Vitamin D and Biochemical parameters with hemodialysis duration. Poster presentation at the 23rd IFCC-EFLM European Congress of Clinical Chemistry and Laboratory Medicine; 2019; Barcelona, Spain.
2. **Begolli G.** The Impact of Hemodialysis duration on Calcium, Phosphorus, and Alkaline Phosphatase Level. Poster presentation at the 33rd World Congress of Biomedical Laboratory Science; 2018; Firenze, Italy.

3. **Begolli G.** Çrregullimi i metabolizmit të Kalciumit dhe Fosforit si dhe fosfatazes alkaline te pacientet ne hemodialize. Poster presentation at the Balkan Clinical Laboratory Meeting (BCLF); 2016; Tirana, Albania.
4. **Begolli G.** Evaluation of inflammatory cytokines and hs-CRP in patients with coronary heart diseases. Poster presentation at the IFCC World Lab; 2014; Istanbul, Turkey.
5. **Begolli G.** Helicobacter Pylori correlation with Lipid Disorders. Poster presentation at the IFCC-EFLM European Congress of Clinical Chemistry and Laboratory Medicine (EuroMedLab); 2013; Milan, Italy.
6. **Begolli G.** Non-surgical periodontal treatment of patients with periodontitis decreases high-sensitivity C-reactive protein levels. Poster presentation at the 7th Conference of European Periodontology; 2012; Vienna, Austria.
7. **Begolli G.** Helicobacter pylori associated with chronic urticaria. Poster presentation at the IFCC-World Lab EuroMedLab; 2011; Berlin, Germany.
8. **Begolli G.** Attitude and practices among youth VCT clients in Kosovo. Poster presentation at the 6-IAS Conference on HIV Pathogenesis, Treatment, and Prevention; 2011; Rome, Italy.
9. **Begolli G.** IgE and RF correlation in synovial fluid and serum in rheumatoid arthritis with reactive synovitis. Poster presentation at the European Immunology Congress; 2009; Berlin, Germany.

Oral Presentation in Scientific activities:

2024: “Inflammation markers in systemic diseases” Kosovo Medical Chamber Congress,

2021: 64th Edition of JIB (Journees de l`inovation en Bioology)

2020: 63th Edition of JIB (Journees de l`inovation en Bioology), 10th Simposium of Institute of Southeast Europe for Health and Social Policy

2019: Significance of systemic inflammatory markers in patients", Turkish Biochemistry Society (TBS) Congress 2020: Antalya, Turkey

2017: "Level of Vitamin D in patient with skin diseases" – 8th International Symposium of Health Sciences – Pristina, Kosovo

2010: The 18 Balkan Clinical Laboratory Federation meeting in Tirana, Albania. "The role of Helicobacter pylori in anemia"

Scientific articles:

1. **Begolli G**, Lukić M, Rumora L, Čorak L, Vukić Dugac A, Jakopović M, Samaržija M, Tomaš I, Knežević J, Debeljak Ž. Serum progastrin-releasing peptide in pneumonia, chronic obstructive pulmonary disease and early-stage primary lung cancers. Biochem Med (Zagreb). 2025 Feb 15; 35(1):010702. doi: 10.11613/BM.2025.010702. Epub 2024 Dec 15. PMID: 39703761; PMCID: PMC11654240.
2. **Begolli G,** Markovic I, Knezevic J, Debeljak Z. Carbohydrate sulfotransferases: a review of emerging diagnostic and prognostic applications. Biochemia Medica. 2023;33:030503. doi: 10.11613/BM.2023.030503.
3. Dalipi Z, Dragidella F, **Begolli G.** Gingival enlargement associated with periodontal destruction and iron deficiency anemia. Int J Med Rev Case Rep. 2021;5(1). doi: 10.5455/IJMRCR.Gingival-enlargement-anemia.

4. Begolli-Stavileci G, **Begolli G,** Begolli L. Interleukin-6, Tumor Necrosis Factor-α, and High-sensitivity C-reactive protein in Diabetic Patients with Helicobacter pylori in Kosovo. Open Access Maced J Med Sci. 2020; 8:172-175. doi: 10.3889/oamjms.2020.3199.
5. **Begolli G**, Shufta V, Veseli S, Budima Basha N, Thaçi S. Correlation of vitamin D and biochemical parameters with hemodialysis duration. Clin Chim Acta. 2019; 493:S462. doi: 10.1016/j.cca.2019.03.977.
6. Valon M, Qyli M, **Begolli G**, Biba A, Ferizi R, Aliko V. Acute toxicity determination of two pharmaceuticals using crab *Carcinus aestuarii* and tadpoles of *Pelophylax kurtmuelleri* cell wellness bioassays. 2017.
7. Begolli L, **Begolli G**, Pajaziti L, Topçiu V, Gafurri Z. *Helicobacter pylori* infection associated with chronic urticaria. J Int Dent. 2014.