# Zyxin expression levels in non-small cell lung cancer patients

## Ekspresija ziksina kod obolelih od nesitnoćelijskog karcinoma pluća

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## Abstract

**Background/Aim.** Non-small cell lung cancer (NSCLC) is the most common cause of cancer-related mortality worldwide. Early detection represents one of the most promising approaches to reduce lung cancer mortality. Zyxin (ZYX) is a member of the focal adhesion protein family, recently identified as a potential early diagnostic marker for NSCLC. The aim of this study was to evaluate ZYX expression levels in NSCLC patients and compare its serum expression profiles between early and advanced clinical stages, different histological subtypes and histological grades. **Methods.** Blood samples were obtained from 90 patients diagnosed with NSCLC in all clinical stages and 30 patients without the clinical and radiological findings and previous history of malignancy. For the quantitative determination of human ZYX concentrations in the serum we used enzyme-linked immunoadsorbent assay (ELISA). **Results**. ZYX exhibited higher serum levels in NSCLC patients as compared to the control samples with exceptionally significant difference ($p = 0.00$). The ROC curve demonstrated a high specificity with AUC = 0.912.

There were no statistically significant differences in the ZYX values between two most common NSCLC types, adenocarcinoma and squamous cell carcinoma ($p = 0.758$). There were no statistically significant differences in the ZYX values among different clinical stages ($p = 0.518$). Only 3 patients had well-differentiated tumor, and no useful data may be extracted from their samples. There were no statistically significant differences in the ZYX values between patients with moderately differentiated tumor and poorly differentiated tumor ($p = 0.48$). **Conclusion.** We found that ZYX was overexpressed in NSCLC, but its expression level was not closely correlated with the tumor size and advanced tumor, node, metastasis (TNM) stage. Our results suggest that ZYX has potential to be an early diagnostic plasma-based tumor marker for NSCLC with the same importance for both adenocarcinoma and squamous cell carcinoma.

**Key words:**
**zyxin; carcinoma, non-small-cell lung; biomarkers, tumor; diagnosis, differential.**

## Apstrakt

**Uvod/Cilj.** Nesitnoćelijski karcinom pluća (engl. *non-small cell lung cancer* – NSCLC) je najčešći uzrok smrti od malignih tumora širom sveta. Rano otkrivanje bolesti najviše obećava u smislu smanjenja smrtnosti od ovog tipa karcinoma. Ziksin (ZYX) je član porodice proteina fokalnih adhezija, nedavno identifikovan kao potencijalni marker za rano otkrivanje NSCLC. Cilj studije bio je procena nivoa ekspresije ZYX kod obolelih od NSCLC i poređenje profila njegove ekspresije u serumu između ranih i odmaklih kliničkih stadijuma bolesti, različitih patohistoloških suptipova i različitih histoloških gradusa tumora. **Metode**. Uzorci krvi dobijeni su od 90 bolesnika sa verifikovanim NSCLC u svim kliničkim stadijumima bolesti i od 30

bolesnika bez kliničkih i radioloških znakova malignoma i bez prethodno verifikovane maligne bolesti. Za kvantitativno određivanje koncentracije humanog ZYX u krvi koristili smo ELISA (eng. *enzyme-linked immunoadsorbent assay*) test. **Rezultati**. Utvrđen je viši nivo ZYX u serumu bolesnika obolelih od NSCLC u poređenju sa kontrolnom grupom, sa izuzetno značajnom razlikom ($p = 0,00$). ROC kriva pokazala je visoku specifičnost testa sa AUC = 0,912. Nije bilo statistički značajne razlike u vrednostima ZYX kod dva najčešća tipa NSCLC, adenokarcinoma i skvamocelularnog karcinoma ($p = 0,758$). Nije utvrđena statistički značajna razlika u nivoima ZYX kod različitih kliničkih stadijuma bolesti ($p = 0,518$). Kod samo tri bolesnika verifikovan je dobro diferentovani tumor, pa nije bilo moguće izvući korisne podatke iz ovako malog uzorka.

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Nije utvrđena statistički značajna razlika u dobijenim vrednostima ZYX kod bolesnika sa srednje diferentovanim i loše diferentovanim tumorom (*p* = 0,48). **Zaključak**. Utvrdili smo da je ZYX prekomerno eksprimiran kod NSCLC, ali nivo ekspresije nije značajnije korelisao sa veličinom tumora, niti uznapredovalim *tumor, node, metastasis* (TNM) stadijumom bolesti. Naši rezultati sugerišu da serumski ZYX ima potencijal kao dijagnostički tumor marker za rano otkrivanje NSCLC, bez obzira da li se radi o adenokarcinomu ili skvamocelularnom karcinomu pluća.

**Ključne reči:**
ziksin; pluća, nesitnoćelijski karcinom; tumorski markeri, biološki; dijagnoza, diferencijalna.

## Introduction

Lung cancer is the leading cause of cancer deaths worldwide. The incidence is approximately 14% in both genders (second after prostate cancer in men and breast cancer in women)[1]. Every year, lung cancer causes more than 1.7 million deaths, more than breast, colon and prostate cancers combined. Lung cancer is classified into two major types: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC is divided into adenocarcinoma (approximately 63%), squamous cell carcinoma (approximately 30%), and large-cell carcinoma (approximately 7%) subtypes, accounting for approximately 85% of all new lung cancer cases[2]. Five-year survival rate of NSCLC still remains < 20%[1]. Most patients are diagnosed in older age ($\cong$ 65 years) and in late-stage (IIIB–IV) where surgical resection is not a standard procedure anymore, according to the guidelines of the American Joint Committee on Cancer (AJCC) showing low overall survival rates at 5 years (5% for IIIB and 1% for IV stages)[3]. However, detection at an earlier stage and treatment by immediate resection are the cornerstones of reducing NSCLC death rates.

Despite the tremendous efforts made to discover blood-based tests for the early diagnosis during the past decades, no tumor markers are available with selectivity to effectively diagnose lung cancer. The most widely used blood-based tumor marker screening includes carcinoembrionic antigen (CEA), cytokeratin 19 fragment (CYFRA 21–1), squamous cell carcinoma antigen (SCCA), and neuron-specific enolase (NSE), without the evidence for their significance in the early diagnosis of lung cancer[4, 5].

Over the past decade, proteomic analysis has become the main tool for investigation of tumor biology. The goal of proteomics is to characterize proteins by evaluation of their expression, functions and interactions, and also may provide information about posttranslational modifications of proteins and evaluate their value as specific disease biomarkers[6]. Any biomarker is defined as a specific that is objectively measured and evaluated as an indicator of normal physiological processes, pathogenic processes and diseases or pharmacological responses to a specified therapeutic intervention[7]. Many studies reported elevation of serum haptoglobin (HP) in NSCLC patients[8, 9], elevation of serum amyloid alpha (SAA)[10, 11] and tissue metalloproteinase inhibitor 2 (TIMP2)[12], and reduction of pigment epithelium-derived factor (PEDF) in the pleural effusion and the serum samples[13], in comparison to controls. Higher levels of leucine-rich alpha-2-glycoprotein (LRG1) were found in urine samples of cancer patients in comparison to healthy subjects[14], and high level of gelsolin expression was significantly associated with death risk of NSCLC patients[15]. Zyxin (ZYX; Uniprot ID, Q15942) showed potential to be used for early diagnosis of NSCLC. Analysis of ZYX values at the different clinical stages demonstrated that the levels of this peptide were alredy elevated at early stages of NSCLC[16].

Zyxin is a zinc-binding phosphoprotein known as a member of the focal adhesion protein family. In normal cells ZYX is involved in cell adhesion, cytoskeleton remodeling[17], stress fibers self-monitoring and repair in response to mechanical stress[18]. But, during mitosis, ZYX also acts as a participant in mitotic control by forming a complex with h-warts/LATS1 on the mitotic apparatus[19]. Zyxin has been already reported as being associated with tumorigenesis. The role of ZYX as a key player in the epithelial-mesenchymal transition (EMT) mechanism[20] and its association to lung cancer as a down regulator of TGF-β inducing cell motility[21] has been recently discussed. Zyxin expression correlates with cancer cell lines with higher malignancy, its activation may play a critical role in regulating yes-associated protein (Yap) activation during tumorigenesis[22]. Upregulation of ZYX in hepatocellular carcinoma had been previously reported[23], and a peptide fragment apparently derived from truncated ZYX has been identified in serum samples from colorectal cancer patients[24]. The expression level of ZYX corresponding to tumorigenesis or tumor mass in the human body has been quite controversial[20].

The purpose of this study was to evaluate ZYX expression levels in NSCLC patients and compare serum ZYX expression profiles between among different histological subtypes and histological grades.

## Methods

A total of 120 patients were recruited from the Clinic for Lung Diseases, Clinical Center Niš, Serbia, between October 2015 and August 2017. Blood samples were obtained from 90 patients diagnosed with NSCLC in all stages and without prior history of other cancers, including adenocarcinoma and squamous cell carcinoma, and 30 patients without the clinical and radiological findings and previous history of malignancy. None of the

patients received chemotherapy, radiotherapy, hormone therapy, or other related antitumor therapies prior recruiting.

The NSCLC patients were classified into clinical disease stages I (n = 9), II (n = 12), III (n = 30) and IV (n = 39) according to the 7th edition of the American Joint Committee on Cancer Tumotr, Node, Metastasis (TNM) staging system [3]. Blood samples were taken prior to surgery for stages I–IIIA and prior to treatment for advanced stage NSCLC patients using serum separator tube. Sera were allowed to clot for two hours at room temperature and then centrifuged at 1000 × g for 15 minutes. Immediately following centrifugation, all specimens were stored at −80°C until being analyzed.

For the quantitative determination of human ZYX concentrations in the serum we used Cusabio Human Zyxin (ZYX) ELISA Kit, Catalog Number CSB-EL027165HU (Cusabio Technology LLC, 8400 Baltimore Avenue, Room 332 College Park, MD 20740, USA). This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for ZYX has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any ZYX present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for ZYX is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of zyxin bound in the initial step. The color development is stopped and the intensity of the color is measured. We created a standard curve by reducing the data using computer software "Curve Expert" capable of generating a four parameter logistic (4-PL) curve-fit. The minimum detectable dose of human ZYX is less than 5.8 pg/mL. Detection range between standards is 23.5–1,500 pg/mL.

This study was approved by the Research Ethics Committee of the Clinical Center Niš. The informed written consents were collected from all eligible patients and the entire study was performed based on the Declaration of Helsinki.

*Introduction to the data and statistical analysis*

The data tracked numerous factors, but the ones relevant for this analysis were merely a dichotomous categorical variable and a continuous variable, specifically whether a patient was diagnosed with lung carcinoma, and what patient's serum level of ZYX was in pg/mL. These two were suffice to establish a link between carcinoma diagnosis and ZYX level. A sample of the data is presented in Table 1.

Once the descriptive statistics was performed, the data were analyzed using simple group-mean comparison. In point of fact, the analysis was conducted using between-groups statistical comparison, with the groups being determined using the dichotomous categorical variable

Dg_Ca representing carcinoma diagnosis. The analysis was conducted using the R programming language, version 3.3.1 built for the x 64 processor architecture, using the following packages: WRS2 [25], effsize [26], and pastecs [27].

**Table 1**

**A sample of the data under observation**

| Number of sample | Diagnosis of cancer (Dg_Ca) | Zyxin concentration (pg/mL) |
|---|---|---|
| 1 | 1 | 773.6667 |
| 2 | 1 | 531.3333 |
| 3 | 1 | 427.4333 |
| 4 | 1 | 484.7667 |
| 5 | 1 | 858.6667 |
| 6 | 1 | 818.0000 |

**Results**

The 90 blood samples were collected from patients who were diagnosed as NSCLC; the average age was 63.56 ± 6.344 years, and the majority of patients were males and smokers. The blood samples from 30 control subjects with other pulmonary diseases were collected as controls by matching their age (62.43 ± 9.380 years), sex, smoking history and their duration and intensity. Patient demographics and clinical profiles are presented in Table 2.

*Descriptive statistics and assumptions of general linear models*

Taken as a whole, the data had an number of 120, and the mean of the variable measuring ZYX concentration was 426.6800277 pg/mL, with a median of 426.2166666 pg/mL and a $\sigma$ of 249.0966559 pg/mL. The large relative value of the standard deviation compared to the mean casted serious doubts on the assumption of globally normal data, and the histogram in Figure 1 shows that the distribution was likely heavy-tailed.
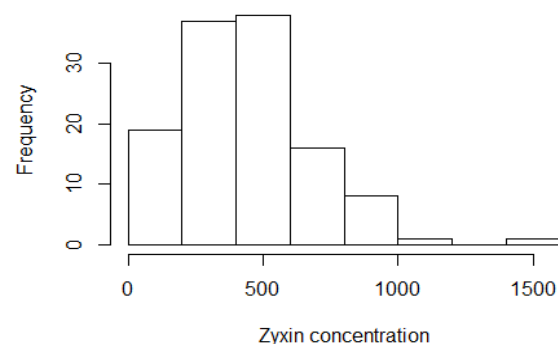


**Fig. 1 – Distribution of zyxin concentrations (in pg/mL) in both group of patients (n = 120).**

However, more crucial information for our purposes was the analysis of the data as split into groups based on

**Table 2**

**Patient demographics and clinical profiles**

| Demographics | NSCLC (n = 90) | Control (n = 30) |
|---|---|---|
| Age (years), mean ± SD (range) | 63.56 ± 6.344 (49–79) | 62.43 ± 9.380 (40–82) |
| Sex, n (%) | | |
|     male | 68 (75.6) | 18 (60) |
|     female | 22 (24.4) | 12 (40) |
| Smoke history, n (%) | | |
|     yes | 76 (84.4) | 23 (76.7) |
|     no | 14 (15.6) | 7 (23.3) |
|     pack-years, mean ± SD | 51.167 ± 30.921 | 44.74 ± 15.235 |
| Clinical histological type*, n (%) | | |
|     adenocarcinoma | 48 (53.3) | |
|     squamous cell carcinoma | 42 (46.6) | |
| Stage**, n (%) | | |
|     IA | 5 (5.6) | |
|     IB | 4 (4.4) | |
|     IIA | 4 (4.4) | |
|     IIB | 8 (8.9) | |
|     IIIA | 17 (18.9) | |
|     IIIB | 13 (14.4) | |
|     IV | 39 (43.3) | |
| Histological grade***, n (%) | | |
|     I | 3 (3.3) | |
|     II | 37 (41.1) | |
|     III | 12 (13.3) | |
|     unknown | 38 (42.2) | |

**\*Histological type of non-small cell lung cancer (NSCLC) according to the 2015 World Health Organization (WHO) histological classification of lung tumors [2]; \*\*Disease stage according to the 7th Edition of the tumor, node, metastasis (TNM) classification of malignant tumors [3]; \*\*\* Histological grade according to the 2015 WHO Classification of Lung Tumors [2]; SD – standard deviation.**

carcinoma diagnosis. To simplify terminology, hence forth the group without the carcinoma diagnosis was referred to as 'control' and the group with the carcinoma diagnosis was referred to as 'effect.' With that said, the descriptive statistics of the control group showed that the number was 30 and that the mean was 168.0501111 pg/mL, while the median was 127.3333334 pg/mL and the $\sigma_D$ 155.5513055 pg/mL. The distribution of the value in the control group can be seen on the histogram in Figure 2.
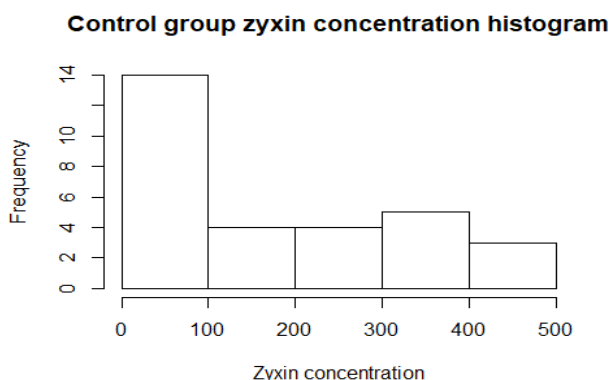
shows. The s-shape to the curve indicates heavy skew which is also evident in the histogram. Similar results were found by applying standard normality tests with the Shapiro-Wilk test allowing us to reject the $H_0$ of normality with a $p$ value of 0.0015193.



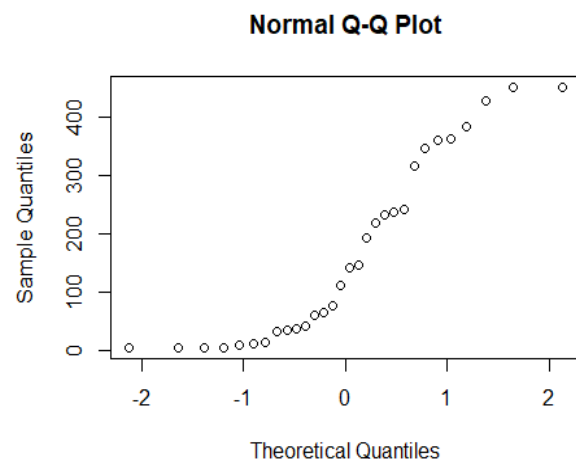**Fig. 2 – Distribution of zyxin concentrations (in pg/mL) in the control group (n = 30).**



**Fig. 3 – The Q-Q plot diagram of mean/median values of zyxin concentrations (in pg/mL) in the control group (n = 30) shows no normality of the data.**

Despite promising mean/median results, this distribution was not normal which the QQ plot in Figure 3

The descriptive statistics of the effect group showed that the number was 90 and that the mean was 512.8899999

pg/mL, while the median was 471.7666667 pg/mL and the $\sigma_D$ was 212.1738935 pg/mL. The distribution of the value with the effect group can be seen on the histogram in Figure 4.

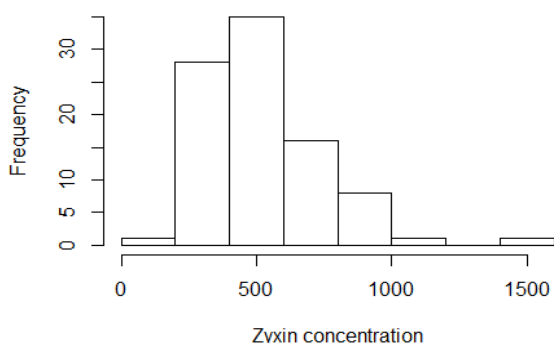**Effect group zyxin concentration histogram**



**Fig. 4 – Distribution of zyxin concentrations (in pg/mL) in the group of non-small cell lung carcinoma (NSCLC) patients (n = 90).**

Despite promising mean/median results, this distribution was not normal which the QQ plot in Figure 5 shows. The deviation from the $\frac{\pi}{4}$ angle to the curve indicated an issue with the kurtosis. This is possible to see by examinging the shape of the histogram suggesting a platykurtic distribution. Similar results were found by applying standard normality tests with the Shapiro-Wilk test allowing us to reject the $H_0$ of normality with a $p$ value of $2.3073684*10^{-5}$.
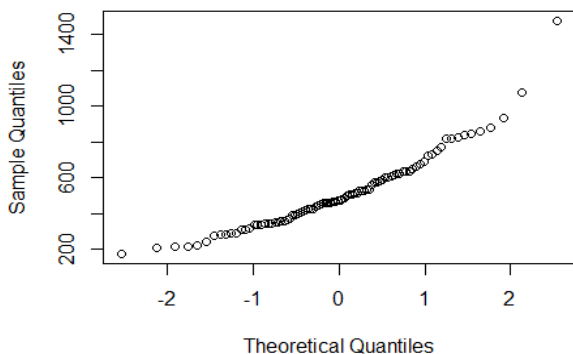
**Normal Q-Q Plot**



**Fig. 5 – Q-Q plot diagram of mean/median values of zyxin concentrations (in pg/mL) in the non-small cell lung cancer (NSCLC) patients (n = 90) shows no normality of the data.**

The groups had unequal sizes, unequal variances, and heavily violated the assumption of normality. With the effect size being what it was, we could confirm that the requirements of the General Linear Model (GLM – of which the $t$-test, normally used in situations like this, is an example) were not met, and, indeed, the requirements of parametric models in general were not met. However, the difference between the means of these two groups was large: more than

double the size, in fact. This means that the effect we were looking for was very large, and thus, we should be able to identify it with great specificity if we apply a statistical technique with sufficient power and sufficiently lax parametric requirements.

*Hypothesis testing for difference in Zyxine level between NSCLC patients and control subjects*

We were dealing with two groups of which one was platykurtic and the other was heavily skewed. Assuming their distributions is equal might influence our results. The alternative was to apply a hybrid approach in which we dealt with each of our issues with GLM assumptions in turn by employing a different solution.

Unequal variances are most simply resolved using the Welch modification of the $t$-test [28]. This test is a modification of the familiar Student's $t$-test (and is commonly employed in its stead in various libraries of statistical software) and pools variances in both populations thus producing an altered test statistic

$$t' = \frac{\overline{Y_1} - \overline{Y_2}}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

and a modified measure of degrees of freedom

$$df' = \frac{(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2})^2}{\frac{s_1^4}{n_1^2(n_1 - 1)} + \frac{s_2^4}{n_2^2(n_2 - 1)}}$$

The remainder of the test is the same. To successfully combine a test that deals with heterogeneity of variances and with a violation of the assumption of normality, a combination of the Welch test and the Yuen modification [29] is necessary. Yuen's approach recreates the Welch test using, instead of means, trimmed means, i.e., means with outlier values on the edges of the distribution clipped to some pre-determined value, generally expressed as a proportion in percent. This level is expressed as $y$.

However, Wilcox [30] suggests that if the group sample sizes are unequal, which was true in our case, a more robust bootstrapped version of the Yuen-Welch is employed. This version of the test uses resampling techniques to estimate the confidence interval for the critical value of the test statistic. This helps reduce the probability of Type I error to the nominal level. The procedure, roughly, proceeds by first computing the trimmed means of the sample and Yuen's estimate of the Squared Standard Errors, like so:

$$d_j = \frac{(n_j - 1)s_{wj}^2}{h_j(h_j - 1)}$$

Then, for the $j$-th group analyzed randomly re-sample with replacement from the available data set $n_j$ observations.

Using the samples generated by this Monte Carlo approach computes the same values as the initial Yuen approach and label them $\overline{X_{i,j}^*}$ and $d_j^*$. Then calculate the following:

$$T_y^* = \frac{(\overline{X_{t1}^*} - \overline{X_{t2}^*}) - (\overline{X_{t1}} - \overline{X_{t2}})}{\sqrt{d_1^* + d_2^*}}$$

This value represents an estimation of the distribution of

$$\frac{(\overline{X_{t1}} - \overline{X_{t2}}) - (\mu_{t1} - \mu_{t2})}{\sqrt{d_1 + d_2}}$$

Repeat the preceding steps generating a sequence of $T_y^*$ values (for our test we selected that this number be $B = 2000$). Then sort these values in ascending order. Let $T_{y(i)}^*$ represent the T-value occupying the i-th place in the sorted array with $i \in [1, B]$. Compute

$$l = \frac{\alpha \cdot B}{2}$$

and round it to the nearest integer and let $u = B - l$.

The confidence interval of $\mu_t$ (being the true mean of the difference in groups) is

$$(\overline{X_{t1}} - \overline{X_{t2}} - T_{y(u)}^* \sqrt{d_1 + d_2}, \overline{X_{t1}} - \overline{X_{t2}} - T_{y(l+1)}^* \sqrt{d_1 + d_2})$$

We used the reference implementation in the WRS2 package with 2,000 resampling steps and a trimming percentage of $y = 20$. The result of this analysis showed a *p*-value of 0, indicating a level to small for the computer to measure, with a test statistic of 7.3819, and a 95% confidence interval for the difference in means of 247.1077 - 437.9487.

This was an exceptionally significant result, showing that we could confidently reject the $H_0$ that the means of the two groups are equal. There was certainly a difference in ZYX concentrations having a significantly larger values in the group diagnosed with NSCLC as compared to the control group of patients without carcinoma diagnosis. Higher plasma levels in NSCLC patients as compared to the control samples are illustrated in Figure 6.

The remaining question was how large the effect was. The traditional approach is to employ Cohen's d effect measure for this purpose, and doing so yields a value of 1.7263458 which corresponds according to Sawilowsky [31] to a very large effect size. However, Cohen's d depends on the same parametric assumptions our data violate. Therefore in accordance to Wilcox and Tian [32], we employ the explanatory measure of effect size, $\hat{\xi}$ which equals 0.889821 and with a 95% confidence interval of 0.8118965 to 0.9635671. The authors of the approach categorize a $\hat{\xi}$ value of 0.5 as a large effect, therefore, we were led to assume that our value represents a very large effect in line with Cohen's d results.
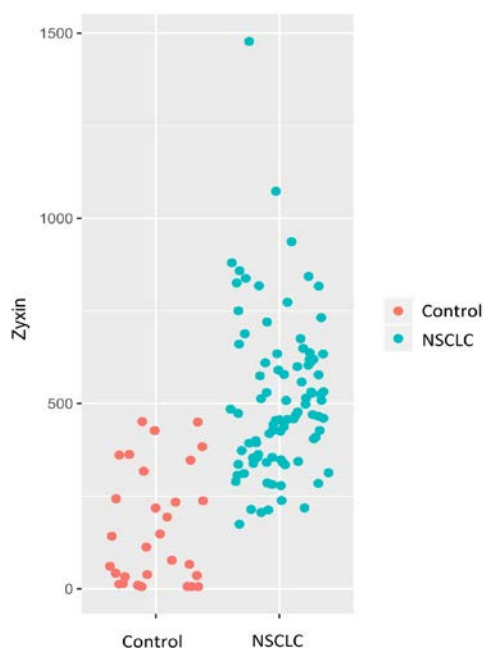


**Fig. 6 – Scatter plots of ELISA results for zyxin in samples of the control group (n = 30) and non-small cell lung cancer (NSCLC) group (n = 90).**

The receiver operating characteristic (ROC) curve generated using ZYX values demonstrated a high specificity toward NSCLC with AUC = 0.912 as shown in Figure 7.
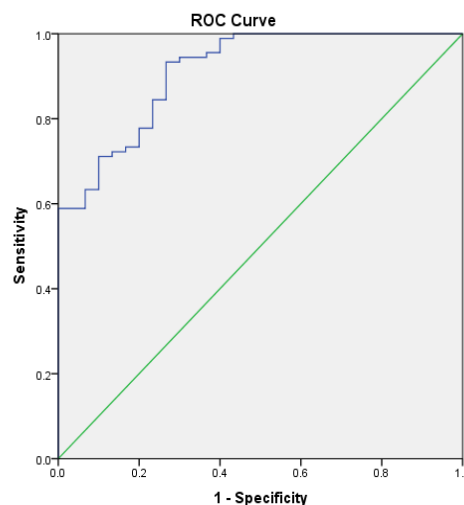


**Fig. 7 – Receiver operating characteristic (ROC) curve for zyxin shows its high specificity toward non-smqall cell lung cancer [area under curve (AUC) = 0.912].**

*Hypothesis testing for difference in Zyxin level among different histological types of NSCLC*

Using the exact method outlined above, it was possible to test for a difference in ZYX levels between groups with one of two most common NSCLC types, adenocarcinoma and squamous cell carcinoma. The analysis showed a test statistic of 0.3114692 with an associated *p*-value of 0.758, showing a difference that was not statistically significant. In practical terms, the actual measured means were 522.1514

pg/mL and 502.3056 pg/mL, respectively, showing the sort of effect only a very large sample size might be able to prove statistically significant difference.

*Hypothesis testing for difference in Zyxin level among clinical disease stages*

Clinical disease stages of NSCLC delineate four separate groups. Demanding a slight change in approach, specifically, instead of using a modified T-test to determine a difference in means between groups, a robust ANOVA-equivalent was used instead, one which tested the hypothesis of equal trimmed means with a $y = 20$, and a $B = 2,000$ without demanding assumptions of normality or heteroscedasticity. Performing this test produced a test statistic 0.8133 and a corresponding *p*-value of 0.518 with an effect size estimate of 0.29. Actual measured means were 443.10 pg/mL for stage I, 562.38 pg/mL for stage II, 439.93 pg/mL for stage III, and 470.10 pg/mL for stage IV. The difference was not large in absolute terms, first, indicating that a larger sample size was required, and second it was not statistically significant, as indicated by the *p*-value. Figure 8 shows a scatter plot of ZYX values for the different clinical stages of NSCLC.
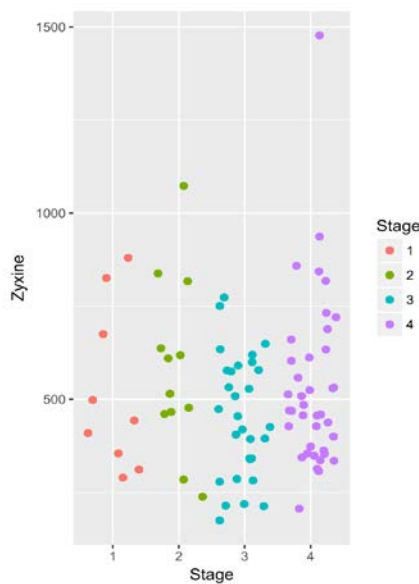


**Fig. 8 – Scatter plots of zyxin values for the different clinical stages of non-small cell lung cancer (NSCLC). Number of samples used in each group are: n = 9 for stage I, n = 12 for stage II, n = 30 for stage III, and n = 39 for stage IV.**

*Hypothesis testing for difference in Zyxin level among histological grades*

Given the nature of histological grades, the decision was made to test only grades II and III, since with applying to the grade of 1, no useful data could be extracted from it (*n* = 3). Using the two-group comparison solution described above, it was possible to test for a difference in zyxin levels between groups with grades II and III. The analysis showed a

test statistic of -0.646 with an associated *p*-value of 0.4845, showing a difference that was not statistically significant. In practical terms, the actual measured means were 522.3604 pg/mL and 546.1500 pg/mL, showing the sort of effect only a very large sample size might be able to prove statistically significant.

**Discussion**

Low-dose computed tomography (CT) screening reduces lung cancer-related mortality, at least for subjects fulfilling the National Lung Screening Trial (NLST) inclusion criteria [33] or under the US Preventive Services Task Force (USPSTF) recommendations [34]. The use of lung cancer predictor models could help defining the subjects with higher risks [35]. In the near future, ongoing research on lung cancer biomarkers could increase accuracy of lung cancer low-dose CT screening. Currently, there are no validated biomarkers for early lung cancer detection.

Kim et al. [16] previously demonstrated that ZYX levels were already elevated at early stages of NSCLC. Their study applied highly multiplexed liquid chromatography-selected reaction monitoring (LC-SRM) assay to verify biomarker candidates in plasma samples for lung cancer, and ZYX was identified as a potential early diagnostic marker for NSCLC.

In this study, we have that zyxin was overexpressed in NSCLC, but its expression level was not closely correlated with tumor size and advanced TNM stage. Zyxin protein exhibited higher serum levels in samples of the NSCLC patients as compared to the control samples with exceptionally significant difference.

The NSCLC group was comprised of serum samples of patients at two different histological types of the disease. Analysis of ZYX values at the different histological types of NSCLC demonstrated equal levels for adenocarcinoma and squamous cell carcinoma. There were no statistically significant differences in ZYX values between two most common NSCLC types. To the best of our knowledge, no studies have investigated the possibility of such differences.

Analysis of the ELISA zyxin values at the different clinical stages of the disaese showed significant elevation in the serum of NSCLC patients already at early stages. This result was consistent with the results from the previous report [16]. There were no statistically significant differences in ZYX values among different clinical stages.

A recent study by Ma et al. [22] has shown that ZYX expression correlates with cancer cell lines with higher malignancy. Zyxin is upregulated in human breast cancer and positively correlates with histological stages and metastasis. We did not establish correlation between degree of differentiation of the tumor and ZYX level due to lack of data for histological grade for 38 (42.2%) of the patients. Only 3 patients had well-differentiated tumor, but no useful data may be extracted from their samples.

There were no statistically significant differences in ZYX values between patients with moderately and poorly differentiated tumor.

### Conclusion

Our results suggest that zyxin fulfilled the criteria for a potential early diagnostic serum-based tumor marker for non-small cell lung cancer, with the same importance for adenocarcinoma and squamous cell carcinoma. Early detection represents a very promising approach to reduce lung cancer mortality. The results of these analyses give us reason to be hopeful, considering high ELISA zyxin values at clinical stages I and II. As one can anticipate, over the coming decade, effective biomarkers in combination with low-dose computed tomography may provide effective tools for the non-small cell lung cancer early detection and improving survival rates in these patients.

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