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Diagnostic value of plasma expression of microRNAs complementary to Drosha and Dicer in lung cancer patients

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Abstract. – OBJECTIVE: Lung cancer (LC) is diagnosed mostly in advanced, non-operable stage, with poor prognosis. The analysis of microRNAs may be a useful tool for early and non-invasive detection of cancer. Dicer and Drosha are enzymes with an essential role for microRNA biogenesis. The aim of our study was to analyze the expression of miRNA-27a-3p, miRNA-31, miRNA-182, miRNA-195 with the ability to reciprocal regulation of Dicer and Drosha expression in lung cancer patients.

PATIENTS AND METHODS: The relative expression of microRNAs was detected by qPCR in plasma of 160 LC patients. The U-Mann Whitney test was used to compare the relative expression between particular groups of lung cancer patients and healthy individuals. The diagnostic value of microRNAs examination was analyzed using a receiver operating curve.

RESULTS: We demonstrated that the plasma levels of miRNA-27, miRNA-31 and miRNA-182 were significantly higher and miRNA-195 significantly lower in the whole group of LC patients and in patients with early stages of NSCLC, in comparison with healthy donors. ROC analysis showed that four studied microRNAs have a potential diagnostic value for early stages of NSCLC with AUC=0.95 for miRNA-27a (94% sensitivity and 81% specificity, *p*=0.0001), 0.71 for miRNA-31 (73% sensitivity and 61% specificity, *p*=0.001) 0.77 for miRNA-182 (70% sensitivity and 79% specificity, *p*=0.0001) and 0.82 for miRNA-195 (74% sensitivity and 80% specificity, *p*=0.0001).

CONCLUSIONS: We have proved that the expression of miRNA-27a-3p, miRNA-31, miRNA-182, and miRNA-195 in patients with LC is different from the expression of these molecules in healthy people. The examination of these microRNAs in plasma could be used in non-invasive lung cancer diagnosis.

Key Words
MiRNAs, Lung cancer, Biomarkers, Drosha, Dicer.

Abbreviations

LC – lung cancer, NSCLC – non-small cell lung cancer, SCLC – small cell lung cancer, miRNA – microRNA, 3'UTR – 3' untranslated region, mRNA – messenger RNA, pre-miRNAs – precursor miRNAs, pri-miRNAs – primary microRNAs, U6 RNA – small nuclear RNA, PCR – polymerase chain reaction, RT-PCR – reverse transcription PCR, qPCR –quantitative PCR, qRT-PCR – quantitative reverse transcription-PCR, ROC – Reciver Operating Curve, AUC – Area Under the Curve, IHC – immunohistochemistry, SSC – squamous cell carcinoma, AAH – atypical adenomatous hyperplasia, SMAD2 – SMAD Family Member 2, SMAD4 – SMAD Family Member 4, TP53 – Tumor Protein 53, EGFR – Epidermal Growth Factor Receptor, HuR – RNA-binding protein that binds to AU-rich fragments in the 3'-UTR of mRNAs, RAS – Ras Proto-Oncogene, MAPK – Mitogen-Activated Protein Kinase, PDCD4 – Programmed Cell Death 4, MYB – MYB Proto-Oncogene, CHEK1 – Checkpoint Kinase 1.

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Introduction

Lung cancer (LC) is the most common cause of cancer deaths in the world. The two main histological types of lung cancer are small cell lung cancer (SCLC, 15-20% of LC cases) and non-small cell lung cancer (NSCLC, 80-85% of LC cases). LC is diagnosed often in advanced, non-operable stages, with poor prognosis. Therefore, the 5-year survival is observed in only 15% of patients[1,2]. This is because of the lack of symptoms in early stages of LC and insufficient treatment effectiveness in advanced disease. Early detection could provide prolongation of survival time and better response to treatment.

Disruption of microRNAs (miRNAs) expression is characteristic for different types of cancers and could be a diagnostic marker for LC. MicroRNAs are small molecules (~21 nt in length). They regulate gene expression on the post-transcriptional level. MiRNAs target the complementary 3'UTR (3' untranslated region) of mRNA and ensure translation repression and/or transcript degradation. MicroRNAs act as products of oncogenes or tumor suppressor genes. Therefore, the expression pattern of miRNAs is characteristic for different kinds of cancers and stages of disease[3].

MicroRNAs genes are transcribed in different parts of nucleus DNA and processed by Drosha enzyme to the precursor miRNAs (pre-miRNAs). Pre-miRNAs are transported to the cytoplasm and processed by Dicer enzyme to a duplex form of miRNAs[4,5,6]. Drosha is a double-stranded RNA-specific endoribonuclease. It is a part of the microprocessor protein complex that acts in the nucleus. It catalyzes the first step of biogenesis of miRNAs which leads to the stem-loop structure form of the primary microRNAs (pri-miRNAs)[6,7]. Dicer is a double-stranded RNA-specific endoribonuclease which process pre-microRNAs into ~21 nucleotide duplex products with an overhang of two nucleotides[6]. Duplexes are incorporated into the RISC complex (RNA-induced silencing complex) and then one of the strands from the duplex binds to the 3'UTR of the targeted mRNA[8].

It has been shown that the expression of Dicer and Drosha enzymes is disrupted in many types of cancers. However, there is limited knowledge about the regulation of miRNAs biogenesis and availability. We assessed the expression of selected circulating miRNAs, complementary to mRNA of Dicer and Drosha. The disrupted expression of these miRNAs could cause, on the basis of the feedback, the formation of different mature microRNAs involved in the regulation of the expression of different genes and in the development of many types of cancers. Therefore, our selected miRNAs could be described as potential biomarkers in cancers (miRNA-27a-3p, miRNA-31, miRNA-182, miRNA-195). The aim of our study is to assess whether the examination of these miRNAs expression has diagnostic value for early and advanced stages of lung cancer.

Patients and Methods

Patients

We enrolled 160 previously untreated lung cancer patients (96 male and 64 female, median age 65 years). The study group contained 22 patients with small cell lung cancer, 57 patients with early stages of non-small cell lung cancer and 79 patients with advanced (67 patients) and locally-advanced (12 patients) non-small cell lung cancer. The characteristic of the groups studied is presented in Table I. The control group consisted of 45 healthy volunteers (17 females and 28 males). The median age of healthy people was 62 ± 5 years.

Isolation of MicroRNA

Blood samples of lung cancer patients and healthy donors were collected into the tubes with K2EDTA and were immediately centrifuged (10 min, 1200 x g, room temperature). The plasma samples were stored at -80°C until isolation of RNA. The total RNA containing miRNA fraction was extracted from 200 µl of plasma. Isolation was conducted with miRNeasy Serum/Plasma Kit (Qiagen, Venlo, Netherlands, Germany) according to the manufacturers' instructions. MicroRNA was stored at -80°C until synthesis of complementary DNA (cDNA). MiRNAs complementary to 3'UTR of Drosha and Dicer1 mRNA were selected using TargetScanHuman 7.0 tool. U6 RNA was used as an internal control.

RT-PCR

Reverse transcription PCR (RT-PCR) was conducted using TaqMan™ MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to manufacturers' instructions. The 15 µl of RT-PCR reaction consists of: 0.15 µl dNTPs, 1 µl MultiScribe™ Reverse Transcriptase, 50 U/µl, 1.50 µl Reverse Transcription Buffer, 0.19

Table I. Characteristic of the studied group (abbreviations: NSCLC – non-small cell lung cancer, NOS – not otherwise specified, SCLC – small cell lung cancer).

Feature	n (%)	
Gender	Male	96 (60)
	Female	64 (40)
Age	Median age	65 (SD=7.32)
	Range	42-83 years
	< 65 years	75 (46.9)
	≥ 65 years	85 (53.1)
Histopathological characteristic	Adenocarcinoma	79 (49.4)
	Squamous cell carcinoma	50 (31.25)
	Large cell carcinoma	2 (1,25)
	NSCLC NOS	7 (4.4)
	SCLC	22 (13.7)
Stage of NSCLC	I	17 (12.3)
	II	27 (19.6)
	IIIA	13 (9.4)
	IIIB	14 (10.1)
	IV	67 (48.6)
Stage of SCLC	Limited disease (stage IIA or IIB)	3 (13.6)
	Extensive disease (stage IIB-IV)	19 (86.4)
Lymph node metastasis	Yes	85 (53.1)
	No	75 (46.9)
Presence of metastases	Yes	105 (65.6)
	No	55 (34.4)
Smoking status	Smokers	114 (71.25)
	Passive-smokers	16 (10)
	No-smokers	9 (5.63)
	No data available	21 (13.12)

µl RNase Inhibitor, 4.16 µl nuclease-free water, 3 µl of specific RT primers, and 5 µl RNA sample. A personal Thermocycler (Biometra, Gottingen, Germany) was used for cDNA synthesis. RT-PCR reactions were conducted in subsequent conditions: 16°C for 30 minutes, 42°C for 30 min, 85°C for minutes and cooling to 4°C.

qPCR

Quantitative real time-PCR was performed using a standard TaqMan® PCR kit protocol on Illumina Eco real-time PCR System (Illumina Inc, San Diego, CA, USA). The 20 µl of PCR mixture contained: 1.33 µl of cDNA, 10 µl of TaqMan® Universal Master Mix II with UNG (Applied Biosystems, Foster City, CA, USA), 1 µl of TaqMan™ MicroRNA Assay (Applied Biosystems, Foster City, CA, USA) and 7.67 µl of nuclease-free water. The numbers of Primers and TaqMan probes used are stated in Table II.

The reaction was conducted in subsequent conditions: 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 s and 60°C for 1 minute. Ct values were obtained, and miRNAs relative expression was calculated using the $2^{-\Delta Ct}$ method.

The statistical analysis was carried out using Statistics 13.1 software (TIBCO Software Inc, Palo Alto, CA, USA). The U-Mann-Whitney test was used to compare miRNAs relative expression between the lung cancer and the control groups, as well as, between different lung cancer subgroups. The Spearman test was used to assess the correlation between the tumor size (in millimetres) or cigarette consumption (pack-years) and

Table II. Catalogue number of qRT-PCT sets used in the assessment of miRNA expression.

RNA	Catalogue No. of qRT-PCR sets
U6 RNA	001973
miRNA-27a	002445
miRNA-31	002279
miRNA-182	002334
miRNA-195	000494

expression of microRNA. The receiver operating curve (ROC) and the area under the curve (AUC) was generated to assess the utility (sensitivity and specificity) of miRNAs expression analysis for LC early diagnosis. In each statistical test *p*-value of < 0.05 was considered significant. The study was approved by the Local Ethical Committee of the Medical University of Lublin (No. KE-0254/218/2015).

Results

The expression of miRNA-27a-3p was significantly higher in the lung cancer group than in healthy volunteers (p=0.0001). Moreover, miRNA-31 and miRNA-182 expression was significantly higher in LC patients than in healthy persons (p=0.002 and p=0.006 respectively). Whereas, the expression of miRNA-195 was significantly lower in the lung cancer group than in the control group (p=0.001) (Figure 1).

Discussion

Dicer and Drosha are enzymes with an essential role in microRNA biogenesis. The expression of different microRNAs varies in the course of neoplastic diseases. Therefore, many authors suppose that the changes in the activity of Drosha and Dicer may be responsible for the changes in the expression of microRNAs. There are several studies exploring the expression of Dicer and Drosha in tumor cells.

In invasive epithelial ovarian cancer, the expression of Dicer and Drosha mRNA corresponded with the protein level and decreased in 60% and 51% of cases[9]. Moreover, patients with high expression of Dicer and Drosha were characterized with increased median survival. There is evidence that in a highly aggressive subtype of breast cancer (triple negative), the expression of Dicer mRNA was significantly decreased while Drosha level was significantly increased in tumors cells compared to non-tumor adjacent tissues[10]. However, the high protein expression of Dicer was found in colorectal cancer (investigated with immunohistochemistry method, IHC). Dicer overexpression predicted poor prognosis for these patients[11].

The expression of Dicer protein (tested with IHC) was lower in advanced lung adenocarcinoma compared to precancerous conditions or *in situ* carcinoma of the lung: the atypical adenomatous hyperplasia (AAH) and *in situ* lepidic form of adenocarcinoma. In part of adenocarcinomas, the Dicer expression was not observed as a result of deletions in the Dicer gene. Moreover, IHC and Western Blot methods showed a higher Dicer level in squamous cell carcinoma (SCC) compared to adenocarcinoma[12].

It can be speculated that the decreased expression of Dicer and Drosha in cancer cells could be the reason for the lower expression of microRNAs acting as tumor suppressors, which promotes the development of cancer.

The expression of Drosha and Dicer is regulated by different microRNAs. There is evidence that Drosha activity is regulated by miRNA-760, miRNA-505-3p, miRNA-30a-5p, miRNA-30d-5p, miRNA-195-3p, and miRNA-27a-3p[13,14,15]. Whereas, 3'UTR of Dicer mRNA contains complementary sequences to miRNA-195-5p, miRNA-31-5p, miRNA-182-5p[16,17,18]. Furthermore, the results of several studies showed that the disrupted expression of various microRNAs could affect the disturbances in the expression of Dicer and Drosha. The complementarity between microRNAs sequences and Dicer and Drosha mRNA sequences are shown in Table III.

MiRNA-27a-3p is the main form of miRNA-27a (miRBase, edition 21). It acts as an oncogene and has been found in a high level in gastric cancer, hepatocellular cancer or glioma[19,20]. Chae et al[21] found high expression of miRNA-27a in squamous cell lung cancer. The authors supposed that miRNA-27a regulates SMAD2 and SMAD4 expression in squamous lung cancer patients as well as in lung cancer cell line. They suggested that this molecule may be a point of action for targeted therapy with antagomirs implementation. Moreover, there are suggestions that the overexpression of miRNA-27a is involved in the resistance to chemotherapy based on cisplatin[24]. On the other hand, disrupted TP53 protein in cancer cells with TP53 gene mutation binds to the promoter of the gene for miRNA-27a and inhibits its expression. It has also been showed that mRNA for epidermal growth factor receptor (EGFR) is a direct target for miRNA-27a[22]. TP53 mutations were identified in 47% of NSCLC, with the highest frequency in squamous cell carcinomas (65%)[23]. Taking together, the disorder of TP53/miRNA-27a/EGFR pathway could be one of many mechanisms involved in the promotion of cell proliferation and tumorigenesis by increasing the expression of EGFR. This is one

Table III. Complementarity between the selected microRNAs sequences and Dicer and Drosha mRNA sequences.

microRNA	Position of 3'UTR in Dicer and Drosha mRNA	Sequences of 3'UTR (top) and mature miRNA (bottom)
miRNA-27a-3p	40-46 of Drosha mRNA	5' ...UUUACUUGCUCAGUAACUGUGAC... 3' CGCCUUGAAUCGG----UGACACUU
miRNA-31-5p	772-778 of Dicer mRNA	5' ...UGCAUAAAAAAGGGUUCUUGCCU... 3' UCGAUACGGUCGUAGAACGGA
miRNA-182-5p	43-50 of Dicer mRNA	5' ...GUCGAAGUUACAGGAUUGCCAAA... 3' UCACACUCAAGAUGGUAACGGUUU
miRNA-195-5p	2608-2614 of Dicer mRNA	5' ...UUAUGAACGCUUUUGUGCUGCUG... 3' CGGUUAUAAAGACACGACGAU

part of limited knowledge about the functions of miRNA-27a in lung cancer.

MiRNA-31 is described as a tumor suppressor. The low expression of miRNA-31 was observed in lung adenocarcinoma with distant metastases and lymph node involvement. Low level of miRNA-31 could be related to the higher ability of tumor cells to proliferation and migration by up-regulation of HuR (RNA-binding protein that binds to AU-rich fragments in the 3'-UTR of mRNAs)[25,26,27]. However, the high expression of miRNA-31 in lung cancer was also described[28]. In this case, the overexpression of miRNA-31 in lung adenocarcinoma was correlated with a decrease in patients' survival. It is assumed that miRNA-31 may play an oncogenic role as a negative regulator of the RAS/MAPK signaling pathway. The high expression of miRNA-31 was related to KRAS gene mutation[29]. This demonstrated that one microRNA could act as an oncogene or as a tumor suppressor depending on histological type or stage of cancer.

MiRNA-182 plays an oncogenic role in cancers. The high expression of miRNA-182 in lung cancer patients was reported[30,31]. It has been shown that higher expression of this miRNA was in lung cancer tissue, as well as LC cell lines, compared to normal tissues and epithelial bronchial cell lines[31]. Furthermore, the high expression of miRNA-182-5p was correlated with the presence of lymph node metastases. Patients with overexpression of miRNA-182-5p had a significantly shorter overall survival compared to those with a low level of this molecule[31]. MiRNA-182 negatively regulates PDCD4 (programmed cell death 4) expression. It has been shown that the overexpression of miRNA-182 was related to cisplatin chemoresistance in NSCLC patients through the down-regulation of PDCD4[32].

MiRNA-195 is described as a tumor suppressor. Su et al[33] have shown that the plasma level of miRNA-195 is downregulated in NSCLC patients compared to the healthy controls. The low expression of miRNA-195 is related to the presence of lymph node metastases and advanced stages of the disease, and it is an independent and unfavorable prognostic factor for NSCLC patients. MiRNA-195 directly binds to the 3′untranslated region of the transcription regulator MYB. *In vitro* and *in vivo* assays showed that a decreased level of this microRNA leads to the overexpression of MYB proto-oncogene in NSCLC cells. This caused an increase in cells proliferation, migration, and invasion. The authors suggested that miRNA-195/MYB axis might be a potential therapeutic target for NSCLC[34]. Another target for miRNA-195 is CHEK1 (Checkpoint Kinase 1) mRNA. The low expression of miRNA-195 was associated with a high expression of CHEK1, which promoted cell proliferation, migration, and invasion. This was related to a shortening of the overall survival in NSCLC patients[35].

We tested the four microRNAs described above in a new context for the involvement of these molecules in the regulation of Dicer and Drosha endonucleases expression. A reciprocal

regulation of the activity of the enzymes involved in microRNAs biogenesis by microRNAs itself has not been considered in the literature. We showed that miRNA-27a-3p, miRNA-31, and miRNA-182 was up-regulated and miRNA-195 was downregulated in lung cancer patients compared to the healthy donors. Thus, the overexpressed miRNA-27a-3p, miRNA-31, and miRNA-182 could contribute to the reduction of Dicer and Drosha activity and to the reduction of the expression of microRNAs with the tumor suppressor activity. However, in this case, the expression of microRNAs with oncogenic activity should also decrease. Possibly, oncogenic and tumor suppressor microRNAs expression change depending on cancer progression. We pointed out that the expression of miRNA-27a-3p and miRNA-182 was slightly lower in patients with advanced stages of NSCLC in comparison to patients with early stages of NSCLC (Figure 2). These controversies require clarification in subsequent studies and it is an attractive direction for further scientific researches. Moreover, the analysis of the expression of mentioned microRNAs (and others juxtaposed in panels) may be a useful tool in early and non-invasive diagnosis of neoplastic diseases[36-40].

Park et al[39] showed an increased expression of miRNA-27a-3p in gastric cancer tissues and plasma samples comparing to healthy individuals. The examination of miRNA-27a showed 75% sensitivity and 56% specificity for gastric cancer diagnosis (AUC=0.7). In our study, we showed for the first time that high expression of miRNA-27a-3p discriminated lung cancer patients from healthy people with 89% sensitivity and 77% specificity (AUC=0.90) (Figure 3). More importantly, the analysis of miRNA-27a-3p expression could be used for diagnosis of early stages of NSCLC (AUC=0.95, 94% sensitivity, and 81% specificity) and for diagnosis of SCLC (AUC=0.90, 91% specificity, and 76% sensitivity). Certainly, the examination of miRNA-27a has the greatest potential diagnostic value for lung cancer diagnosis.

Ma et al[41] conducted a meta-analysis of 12 studies concerning miRNA-31 analysis in cancer diagnosis. The authors indicated that the high expression of circulating miRNA-31 had a great value for the diagnosis of cancers (AUC=0.79, 95% CI: 0.73-0.86). Zhu et al[42] indicated that the serum levels of miRNA-182, miRNA-183, and miRNA-210 were significantly up-regulated and the miRNA-126 level was significantly downregulated in NSCLC patients compared to the healthy controls. The examination of the serum level of miRNA-182 had a sensitivity of 63% and a specificity of 80% to differentiate NSCLC patients from healthy controls (AUC of 0.73)[42]. In our study, miRNA-31 and miRNA-182 expression was significantly higher in the early stages in NSCLC patients compared to healthy people[28]. The examination of miRNA-31 and miRNA-182 could discriminate patients with an early stage of NSCLC and healthy people (AUC=0.71 with 73% sensitivity and 61% specificity for miRNA-31 and AUC=0.77 with 70% sensitivity and 79% specificity for miRNA-182) (Figure 4).

Su et al[33] indicated that the plasma miRNA-195 expression is lower in NSCLC patients than in healthy persons. The authors suggested that miRNA-195 is a useful marker for NSCLC diagnosis (AUC=0.89 with 79% sensitivity and 86% specificity), especially for the early stages of NSCLC (patients in stage I or II had a higher level of miRNA-195 than patients with stage III of NSCLC). Our results found that the expression of miRNA-195 is low in patients with NSCLC and also in patients with SCLC. The examination of miRNA-195 expression may be a diagnostic tool for non-invasive detection of the early and advanced stages of NSCLC and SCLC. The analysis of miRNA-195 expression detected the early stages of NSCLC with 74% sensitivity and 80% specificity (AUC=0.82).

Conclusions

There are several studies about the disturbances of microRNAs expression in cancers with well-proven diagnostic and prognostic value for cancer diagnosis. Our investigation concerned the examination of microRNAs with the ability to regulate the activity of Dicer and Drosha enzymes involved in microRNAs biogenesis. *We have proven* that the expression of miRNA-27a-3p, miRNA-31, miRNA-182, and miRNA-195 in patients with lung cancer is different from the expression of these molecules in healthy people and that the examination of these microRNAs in plasma could be used in non-invasive lung cancer diagnosis.

Conflict of Interests

The Authors declare that they have no conflict of interests.

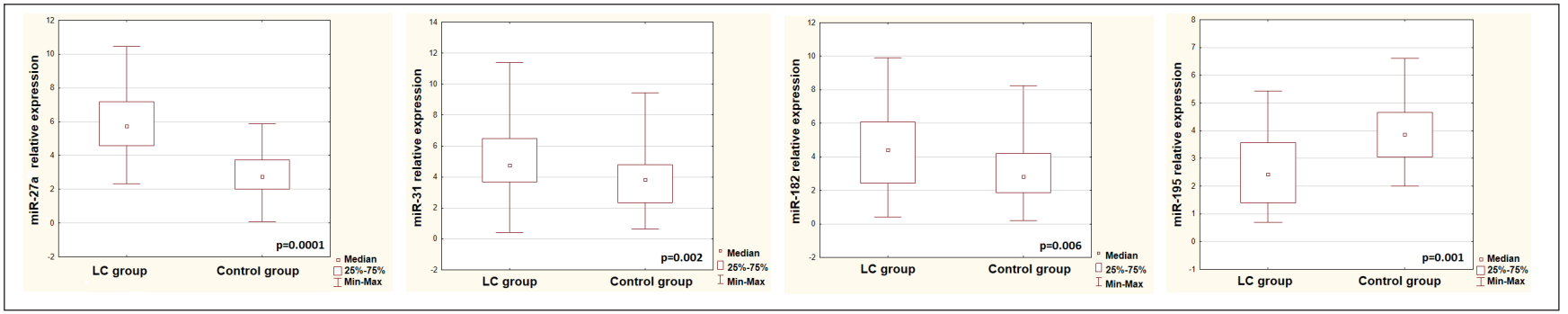

Figure 1. Relative expression of miRNA-27a, miRNA-31, miRNA-182, miRNA-195 in plasma samples of lung cancer patients (LC group) compared with healthy donors (Control group). Patients with early stages (I-IIIA) of NSCLC showed significantly higher expression of miRNA-27a-3p (p=0.0001), miRNA-31 (p=0.003), and miRNA-182 (p=0.0003) than healthy persons. The expression of miRNA-195 was significantly lower in the early stages (I-IIIA) NSCLC patients than in healthy donors (p=0.0001). The expression of miRNA-27a-3p (p=0.001) and miRNA-31 (p=0.0007) was significantly higher, and the expression of miRNA-195 (p=0.007) was significantly lower in advanced NSCLC patients (IIIB – IV stages) in comparison to healthy donors. There were no differences in the expression of miRNA-182 between advanced NSCLC patients and healthy persons (p=0.5) (Figure 2).

We found a significantly higher expression of miRNA-27a-3p (p=0.0001) and lower expression of miRNA-195 (p=0.0001) in SCLC patients than in healthy volunteers. The expression of miRNA-31 and miRNA-182 was similar in SCLC patients and healthy people (p=0.3 and p=0.1, respectively). We did not find any significant differences in miRNAs between the group of NSCLC and SCLC patients (p=0.4 for miRNA-27a-3p, p=0.2 for miRNA-31, p=0.7 for miRNA-182 and p=0.2 for miRNA-195). Moreover, miRNAs expression was similar in patients with different histological types of NSCLC.

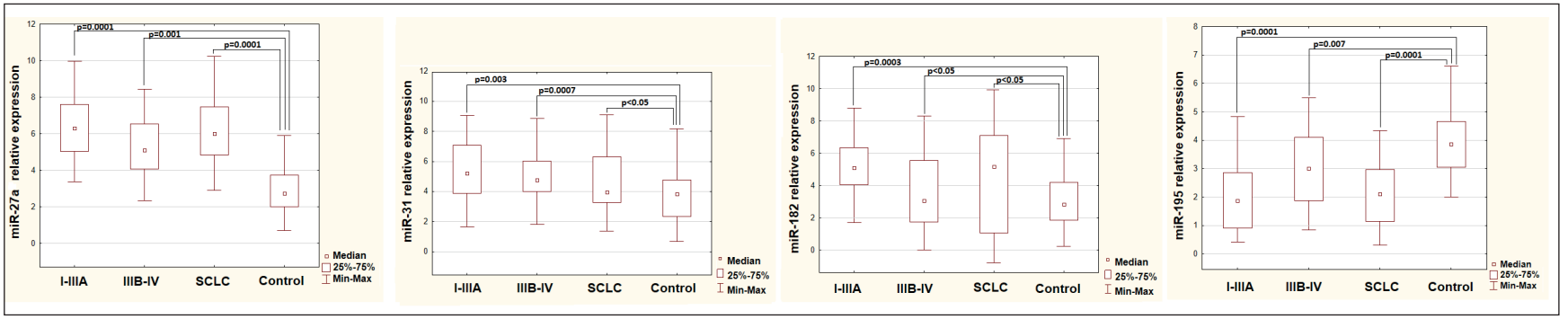

Figure 2. Comparison of miRNA-27a, miRNA-31, miRNA-182, miRNA-195 expression in early stages (I-IIIA) and advanced stages (IIIB-IV) of NSCLC, as well as in SCLC, to healthy donors (Control). We found a significantly lower expression of miRNA-195 in LC patients with mediastinal lymph node metastases in comparison to patients without lymph node enlargement (p=0.005). The presence of distant metastases had no effect on miRNAs expression. There was no correlation between the expression of miRNAs and tumor size. Demographic factors had a moderate influence on the expression of miRNAs in NSCLC and SCLC groups. We showed a higher expression of miRNA-182 in lung cancer patients under 65 years old (p=0.02) in comparison to older LC patients. Moreover, the expression of miRNA-182 and miRNA-195 was significantly higher in female than in male LC patients (p=0.001 and p=0.002, respectively). The smoking status had no effect on the expression of examined miRNAs. There was no correlation between miRNAs expression and cigarettes consumption measured by pack-years. In our further analysis, we generated receiver operating curves (ROC) to evaluate the utility of miRNAs expression examination in discrimination of lung cancer patients and healthy people. The area under the curve (AUC) for miRNA-27a-3p was 0.90 (95% confidence interval (CI): 0.86-0.96) with 89% of sensitivity and 77% of specificity (p=0.0001). AUC for miRNA-31 was 0.63 (95% CI: 0.59-0.77) with 68% of sensitivity and 64% of specificity (p=0.0001). AUC for miRNA-182 was 0.64 (95% CI: 0.55-0.72) with 51% of sensitivity and 71% of specificity (p=0.002). AUC for miRNA-195 was 0.76 (95% CI: 0.69-0.83) with 74% of sensitivity and 71% of specificity (p=0.0001). The ROC curves are shown in Figure 3.

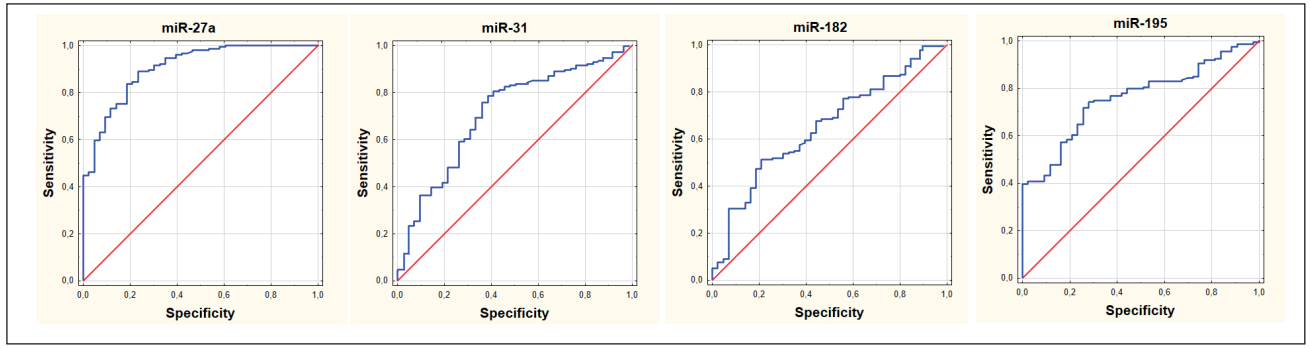

Figure 3. The receiver operating curves for the analysis of the expression of miRNA-27a-3p, miRNA-31, miRNA-182, and miRNA-195 used to discriminate lung cancer patients and healthy people. Furthermore, we generated the ROC to evaluate the utility of miRNAs expression examination in discrimination of the early stages of NSCLC patients and healthy persons. The AUC for miR-27-3p was 0.95 (95% CI: 0.9-0.99) with 94% sensitivity and 81% specificity (p=0.0001). The AUC for miRNA-31 was 0.71 (95% CI: 0.61 - 082) with 73% sensitivity and 61% specificity (p=0.001). The AUC for miRNA-182 was 0.77 (95% CI: 0.68-0.87) with 70% sensitivity and 79% specificity (p=0.0001). The AUC for miRNA-195 was 0.82 (95% CI: 0.74-0.90) with 74% sensitivity and 80% specificity (p=0.0001). The ROC for this analysis is showed in Figure 4.

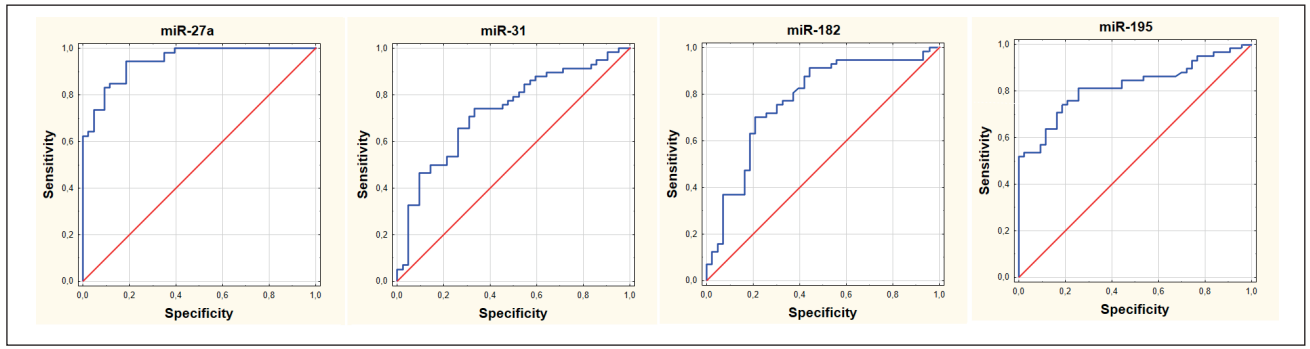

Figure 4. ROC for the analysis of the expression of miRNA-27a, miRNA-31, miRNA-182, and miRNA-195 used to discriminate the early stages of NSCLC patients and healthy persons. The analysis of sensitivity and specificity of miRNAs expression assessment was also performed for SCLC patients. The AUC for miRNA-27a-3p was 0.90 (95% CI: 0.83-0.98) with 91% specificity and 76% sensitivity (p=0.001). The AUC for miRNA-195 was 0.71 (95% CI: 0.58-0.84) with 96% sensitivity and 23% specificity (p=0.002). The statistical analysis did not provide significant results for miRNA-31 and miRNA-182 (p=0.13 and p=0.15, respectively).

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