Original Article

Altered carnitine transporter genes (*SLC22A5*, *SLC22A16*, *SLC6A14*) expression pattern among lung cancer patients

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Background: Despite the decrease of morbidity rate of non-small cell lung cancer (NSCLC) in recent years, it is still a cancer with poor prognosis. Lung cancers (LCs) are usually diagnosed at a late stage of the disease due to non-specific clinical symptoms. Proper regulation of carnitine levels is important in the context of development and increased risk of cancer cells proliferation. The expression profiles and clinical value of *SLC* family members in LC remain largely unexplored. The aim of the study was the assessment of *SLC22A16*, *SLC22A5* and *SLC6A14* mRNA expression level among patients suffering from NSCLC. The obtained results were compared with the clinical and the pathological features of NSCLC patients.

Methods: Through reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and bioinformatics studies, the evaluation of carnitine transporting genes (*SLC22A16*, *SLC22A5* and *SLC6A14*) mRNA levels was performed in order to elucidate their connection to clinical features of patients and influence on overall survival (OS).

Results: The analysis showed a significant difference for the *SLC22A5* gene of NSCLC patients and for *SLC6A14* and *SLC22A5* genes in LUSC patients in terms of sex (P=0.002, P=0.02 and P=0.001, respectively) and in terms of tobacco smoking (P=0.04). Analysis also revealed a significant negative correlation for *SLC22A5* and *SLC22A16* genes expression level in the lung adenocarcinoma (LUAD) subtype with standardized uptake value (SUV) (r=–0.40, P=0.02 and r=–0.43, P=0.04). The significant downregulation of gene expression compared to normal adjacent tissue was observed for *SLC22A5* in lung squamous cell carcinoma (LUSC) and for *SLC6A14* in both LUAD and LUSC subtypes. The effect of the *SLC22A5*, *SLC22A16* and *SLC6A14* gene expression at the time of diagnosis on the OS time of LC patients revealed that lower expression correlated with a shorter 5 years OS (all P values <0.01). The effects were distinct after division for LUAD and LUSC subtypes.

Conclusions: The expression levels of genes encoding carnitine transporters are diverse, hinting at a potentially altered carnitine metabolism in LC patients. Notably, this variance is not uniform and exhibits specificity across LC subtypes, with marked distinctions between LUAD and LUSC. The correlation between gene expression levels and OS of patients underlines the prognostic significance of *SLC* genes within these cancer subtypes.

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Introduction

Background

Despite the continuous progress in medicine, the lung cancer (LC) is still the most common cause of cancer-related death worldwide. LC is the second most frequently diagnosed cancer among men and third in the group of women in the polish population (1). There are many factors that predispose to LC development such as smoking, excessive alcohol consumption or the influence of environmental and biological factors (2,3).

LC is a malignant tumor originating from the epithelium of the respiratory tract with a high variability so it can lead to many different histological subtypes. Clinically, primary LCs can be divided into two groups: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC accounts for 80% of all diagnosed LCs. There are three major subtypes of NSCLC: squamous cell carcinoma [SCC, lung squamous cell carcinoma (LUSC)], adenocarcinoma [AC, lung adenocarcinoma (LUAD)] and large cell lung carcinoma (LCC). NSCLC is a type of LC that can be resistant to chemotherapy, but is susceptible to surgical treatment (about 75–80% of cases), radiotherapy and biological therapies in case of specific gene mutations occurrence (4-8). Despite the decrease of morbidity rate of NSCLC in recent years (especially among the male population), NSCLC is still a cancer with poor prognosis. LC are usually diagnosed at a late stage of the disease due to non-specific clinical symptoms (1,2,8,9).

Researchers are still looking for new alternative forms of early detection of this disease, especially among various genetic factors such as: suppressors or mutator genes. The molecular features are important in the assessment of the patient's prognosis as well as in the selection of appropriate targeted therapy. In the group of suppressors, which are downregulated in NSCLC, we can distinguish *FHIT* (50–75% of cases), *RARB* (40% of cases), *TP53* (42% of cases), and *CDKN2A* (60% of cases) genes (10-12). It is worth to mention that chromosomal translocations of *EML4* and *ALK* genes lead to the abnormal EML4-ALK protein production, which has a negative predictive value in NSCLC patients (10,11,13). On the other hand, in NSCLC an increase expression level of mutators genes such as: *EGFR* (43–89% of cases), *KRAS* and *BRAF* (mainly in patients with AC) was observed (10-12). Aforementioned genes are successfully targeted by biological treatment and in recent years improved the cure rate and survival of NSCLC patients. Moreover, the increased expression of the *TERT* gene (which is caused by the loss of heterozygosity) occurs in patients with non-small cell carcinoma and may be a potential therapeutic target (14).

Highlight box

Key findings

- The expression level of genes encoding carnitine transporters are altered in lung cancer patients and exhibits specificity across lung adenocarcinoma and lung squamous cell carcinoma subtypes.
- The correlation between gene expression level and overall survival of patients underlines the prognostic significance of *SLC* genes.

What is known and what is new?

- Proper regulation of carnitine levels is important in the context of development and increased risk of cancer cells proliferation.
- Assessment of *SLC22A16*, *SLC22A5* and *SLC6A14* mRNA expression level among patients suffering from non-small cell lung cancer and its connection to clinicopathological features and patients' survival.

What is the implication, and what should change now?

- Given the complexity and heterogeneity of lung cancer, further investigations into the SLC gene family's role are crucial. Future research should focus on unraveling the intricate mechanisms through which these transporters influence cancer metabolism, resistance to therapy, and patient outcomes. Also, their usage as potential prognostic factors should be evaluated.

Rationale and knowledge gap

Some recent studies were also focused on the role of carnitine transporters in the LC development. Carnitine is a chemical compound with a betaine structure, which play crucial role in transport of fatty acids between cytoplasm and

mitochondria (14). Biosynthesis of carnitine occurs mainly in the liver, kidneys and in the brain. Proper concentration of carnitine is responsible for homeostasis maintaining in the organism (14,15). Carnitine is an important acyl group carrier during fatty acid oxidation (FAO) process. Activated acyl groups (arising through the conversion of carnitine to acyl carnitine by acetyltransferase isoenzymes) can be transferred to coenzyme A (CoA) to function as a rapidly available energy source via FAO. Consequently, carnitine is involved in the regulation of acyl-CoA/CoA levels and is related to metabolic and energy regulation of the body (16,17). Moreover, the one of the most efficient pathways producing energy in cells where participation of carnitine is necessary is the process of β-oxidation of fatty acids taking place in the mitochondria (16). The processes of carcinogenesis are characterized by high-energy demand. Rapidly dividing cancer cells require large amounts of energy to maintain replication and proliferation. It has also been shown that together with the growth and the degree of malignancy of the tumor the energy demand increases (16,18). Cancer cells regulate the energy demand, among others, through the process of β-oxidation of fatty acids (19-21). Prostate cancer or diffuse large B-cell lymphoma are characterized by high carnitine demand and use the FAO as a main source of energy for cancer cells (22-24). Therefore, proper regulation of carnitine levels is important in the context of development and increased risk of cancer cells proliferation. It is worth to mention that carnitine is transported between tissues by membrane transporters from the family of SLC proteins. There are three main carnitine transport proteins that are found in the plasma membrane and that deliver carnitine to the cells. These include SLC22A16, SLC22A5 and SLC6A14 (25,26).

The SLC22A5 protein (encoded by the *SLC22A5* gene) is a multi-specialty transporter of organic cations, many drugs and environmental toxins. It is one of the main membrane transporters in the carnitine pathway and is involved in active cellular carnitine uptake. The *SLC22A5* gene is located on chromosome 5 of the q31.1 locus and consists of 11 exons. It has been shown that this gene participates in the transport of oxaliplatin and other chemotherapeutic drugs, therefore it may become a potential therapeutic target among cancer patients (27-30).

The *SLC22A16* gene is located on chromosome 6, locus q21. The expression of this gene has been found in the large intestine, testes, liver, kidneys, heart or lung tissue. *SLC22A16* gene encodes an organic protein which transports ions and carnitine. The SLC22A16 protein is also a carnitine transporter with high affinity for sodium ions (transport dependent on sodium ions and alkaline pH). According to source data, healthy tissues are characterized by a relatively low expression level of the *SLC22A16* gene. In the case of cancer cells, the expression of *SLC22A16* is increased (16,31-34).

The *SLC6A14* gene belongs to the *SLC6* gene sub-family and is located on the X chromosome locus q23. This gene encodes the SLC6A14 protein, which belongs to the group of plasma membrane transporters. This protein is mainly responsible for the transport of amino acids, neurotransmitters and osmolytes through the cells. Data suggested that in comparison to the healthy tissues *SLC6A14* gene is overexpressed in cancer cell (35-39).

Objective

The aim of the study was the assessment of *SLC22A16*, *SLC22A5* and *SLC6A14* mRNA expression level among patients suffering from NSCLC. The obtained results were compared with the clinical and the pathological features of NSCLC patients such as: age, sex, smoking status, histological type, TNM classification, grading, standardized uptake value (SUV), hemoglobin level, previous cancer history. We present this article in accordance with the STROBE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-448/rc).

Methods

Patient recruitment

113 patients (48 women and 65 men, mean age 69 years) diagnosed with NSCLC were enrolled in the study. All recruited patients were diagnosed between 2022–2023 at the Department of Thoracic Surgery, General and Oncological Surgery of the Medical University of Lodz (Lodz, Poland). Inclusion criteria for the study comprised: diagnosis of LC (ICD-10: C34), age from 18 to 99 years, ability to give informed consent to participate in the study, admission for the purpose of tumor removal surgery, data availability of tumor staging and tobacco smoking (with pack years). This study was conducted in compliance with the principles of the Helsinki Declaration (as revised in 2013). The Ethics Committee of Medical University of Lodz approved the present study (protocol numbers: RNN/87/16/KE and KE/952/22). Informed consent was obtained from all patients prior to their inclusion in the

Table 1 Clinical and pathological characteristics of patients

Clinical feature	Values
Sex, n	
Men	65
Women	48
Age, years, mean ± SD	69.5±7.97
Tobacco smoking, n	
Yes	72
No	41
Pack-year, mean ± SD	39.9±24.7
Histological subtype, n	
Adenocarcinoma	38
Squamous cell carcinoma	49
Large cell carcinoma	8
Other (including NSCLC NOS)	18
Primary tumor size (T in TNM classification), n	
T1	33
T2	39
T3	22
T4	19
Lymph node involvement (N in TNM classification), n	
N0	41
N1	48
N2	18
N3	3
Nx	3
Presence of metastasis (M in TNM classification), n	
M0	50
M1	8
Mx	55
Stage, n	
I	30
II	37
III	39
IV	7
Hemoglobin, g/dL, mean ± SD	13.03±1.69
SUV, mean ± SD	14.69±8.89
Previous cancer history, n	
Yes	23
No	90
Cancer occurrence in the family, n	
Yes	9
No	104

SD, standard deviation; NSCLC, non-small cell lung cancer; NOS, not-otherwise specified; TNM, tumor, node, metastasis; SUV, standardized uptake value.

study. All data collected in the study were anonymous. All demographic, clinical and pathological characteristics of patients are presented in *Table 1*.

Gene expression level assessment

In order to assess the expression level of selected genes from the *SLC* family, the RNA was isolated from tissue specimens taken during operations from patients diagnosed with NSCLC. The RNA extraction was performed using a Total RNA Mini kit (A&A Biotechnology, Gdynia, Poland), according to the manufacturers' protocol. The purity and concentration of RNA samples were assessed nanospectrophotometrically. In all samples, the A60/A280 ratio ranged from 1.8 to 2.0, which proves the good quality of the extraction. Until further analysis, RNA samples were stored at –80 ℃.

The obtained RNA samples were reverse-transcribed into cDNA using a High-Capacity cDNA Reverse Transcription kit (Applied Biosystems; ThermoFisher Scientific, Inc., Waltham, MA, USA), according to the manufacturers' protocol. The temperature parameters used for the RT-PCR reaction were following: 25 ℃ for 10 min, 37 ℃ for 120 min and 85 ℃ for 5 min. The final concentration of obtained RNA in the reaction mixture in all samples was 0.05 µg/µL.

SLC22A16, *SLC22A5* and *SLC6A14* mRNA expression level was assessed using qPCR performed in a CFX 96™ Real-Time PCR detection thermocycler (Bio-Rad, Hercules, California, USA). *ACTB* gene was used as a reference gene. The reaction mixture and primer sequences that are used in each reaction are presented in *Table 2*.

To ensure the quality of the assay in every trial no

Table 2 Conditions of qPCR and primer sequences

Parameters	*SLC22A16*	*SLC22A5*	*SLC6A14*	*ACTB*
Reaction mixture	• 5 µL of iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules, California, USA) • 0.7 µL of 10 µM each primer • Nuclease-free water to final volume of 9 µL • 1 µL cDNA template			
Thermocycling conditions	• Initial denaturation at 95 ℃ for 10 min • 40 cycles of: denaturation at 95 ℃ for 10 s, annealing at 56 ℃ for 1 min, elongation at 72 ℃ for 20 s			
Primers sequences				
Forward primer	AGTTGTTTGTTGGGACTATG	TGTGGATGACCATATCAGTG	AAGGTGTGGGAATTACAATG	GACGACATGGAGAAAATCTG
Reverse primer	CATTCTCTGACTCCAGTTTTG	AAAGGAAGCAGTTCACAAAG	ACAGTTTTTATCTGACCACG	ATGATCTGGGTCATCTTCTC

template control (NTC) samples were included. The investigated and reference genes were amplified in triplicate. A reference gene was used as a normalizer for the correction of gene expression. The means of obtained Ct values for both genes were calculated. Relative changes in gene expression, determined by RT-qPCR analysis, were estimated based on the $2^{-\Delta\Delta Cq}$ method by Livak and Schmittgen (40).

External databases

The selected *SLC* family genes expression level in cancer versus normal tissue was assessed using the Tumor Immune Estimation Resource (TIMER) (https://cistrome.shinyapps.io/timer/, accessed on 12–16 February 2024) on-line tool (41). The Cancer Genome Atlas (TCGA) RNA-seq database was chosen with the DiffExp module for calculating the differential expression between tumor and adjacent normal tissues for *SLC22A16*, *SLC22A5* and *SLC6A14* genes. Data are presented as individual box-plots; statistical significance was estimated by the Wilcoxon test.

The Kaplan-Meier plotter (https://kmplot.com/analysis/, accessed on 12–16 February 2024) (42) was utilized to associate overall survival (OS) between two groups of patients (low *vs.* high gene expression level) for *SLC22A16*, *SLC22A5* and *SLC6A14* genes in LC, LUAD and LUSC subtypes. The threshold for OS calculation was set for 60 months (5 years) with the "autoselect best cutoff" selection, without biased arrays. The log-rank test was used for P value calculations.

Statistical analysis

Statistical analysis was performed using Statistica 13.1 software (TIBCO, Palo Alto, CA, USA). The Shapiro-Wilk W test was used to verify the compliance of the examined quantitative characteristics with the normal distribution. Where normal distribution was achieved, the results are presented as the mean with standard deviation (SD), in the case of non-compliance with the normal distribution, the results are presented as the median and interquartile range (IQR). Homogeneity of variances was assessed using Levene's test. To assess the significance of differences in quantitative data between groups The Student's *t*-test, Mann-Whitney *U* test, Kruskal-Wallis test and analysis of variance (ANOVA) were applied. Pearson's r correlation was used to search for associations between two quantitative features. For the purpose of statistical analyses, where numbers of individuals were small, some subgroups of patients were joined together, e.g., patients with tumor stage III and stage IV, lymph node involvement N2 with N3. In all calculations, a P value ≤ 0.05 was considered statistically significant, unless otherwise specified. Where applicable, multiple testing correction was applied with a Benjamini-Hochberg method, FDR value was set at 0.05. The adjusted P values are presented as Q-values.

Results

Wet analysis

In the first stage, an analysis was carried out in a group of

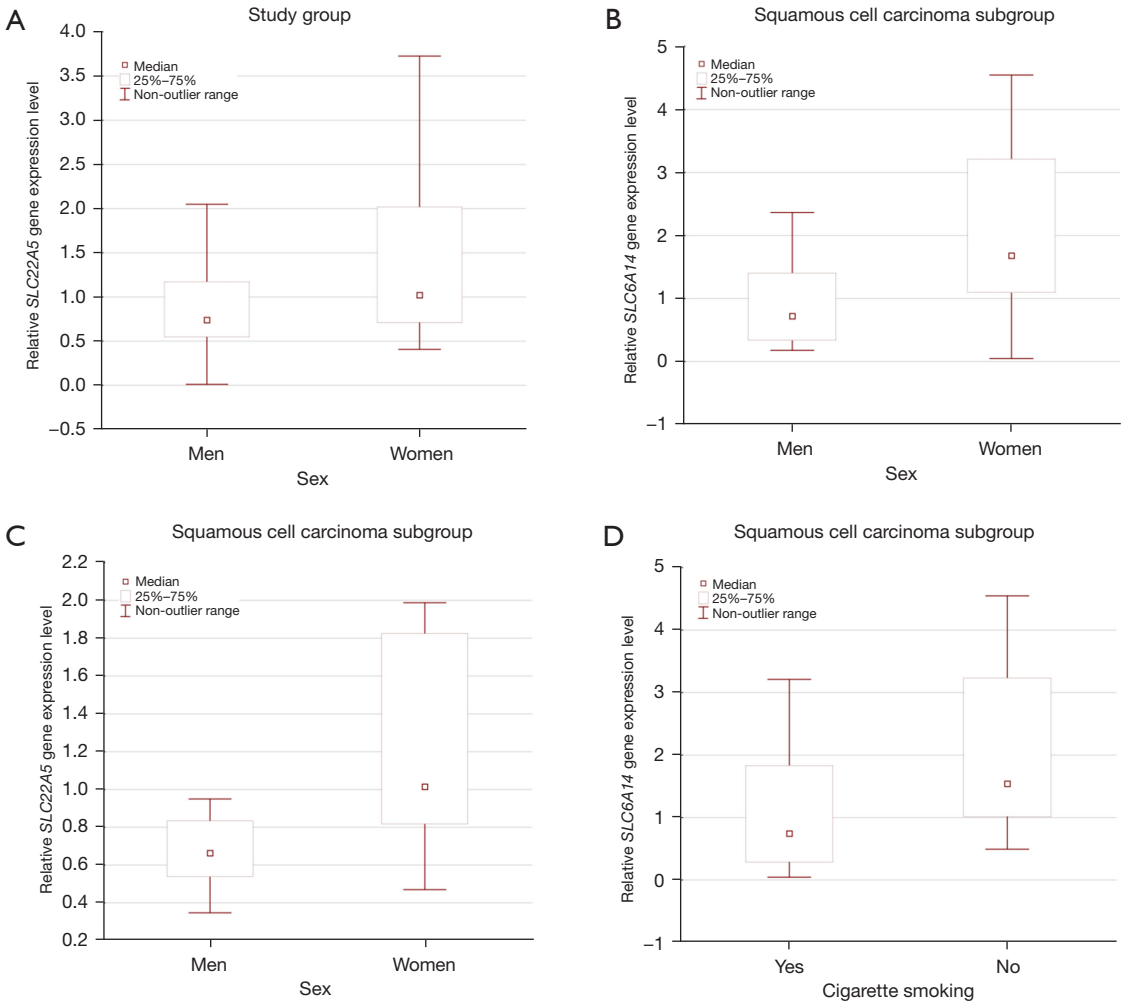

Figure 1 Differences in observed relative expression levels of selected *SLC* genes in terms of sex [(A) *SLC22A5* gene; (B) *SLC6A14*, LUSC subtype; (C) *SLC22A5* gene, LUSC subtype] and cigarette smoking [(D) *SLC6A14* gene, LUSC patients]. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

patients with LC in terms of a number of clinicopathological features.

The analysis of the relative expression level of selected genes from the *SLC* family in the study group showed a significant difference for the *SLC22A5* gene in terms of sex. Higher levels were observed in women (P=0.002, Q=0.01, *Figure 1A*). Since NSCLC is a group of heterogeneous disorders and recent research suggests that individual subtypes differ at the molecular, pathological and clinical levels and should be classified and treated as distinct entities, the analysis was also performed comparing individual subtypes. A significant difference was found for *SLC6A14* and *SLC22A5* genes in LUSC patients. Here again, higher values were observed in women (P=0.02, Q=0.02, *Figure 1B* and P=0.001, Q=0.004, *Figure 1C* accordingly). In the LUAD subtype, the analysis did not show any significant differences (P=0.18 and P=0.24 accordingly).

Differences in observed expression levels were then examined in terms of cigarette smoking. In the entire study group and divided by sex (men/women), no significant differences were found for *SLC22A5* (P=0.55, P=0.93 and P=0.62), *SLC22A16* (P=0.56, P=0.09 and P=0.50) and *SLC6A14* (P=0.72, P=0.09 and P=0.25); after division into LC subtypes, a significant difference was found for the *SLC6A14* gene in the subgroup of patients with LUSC (P=0.04, Q=0.04, *Figure 1D*). Active smokers had lower relative levels of *SLC6A14* gene expression.

Furthermore, analysis revealed a significant negative

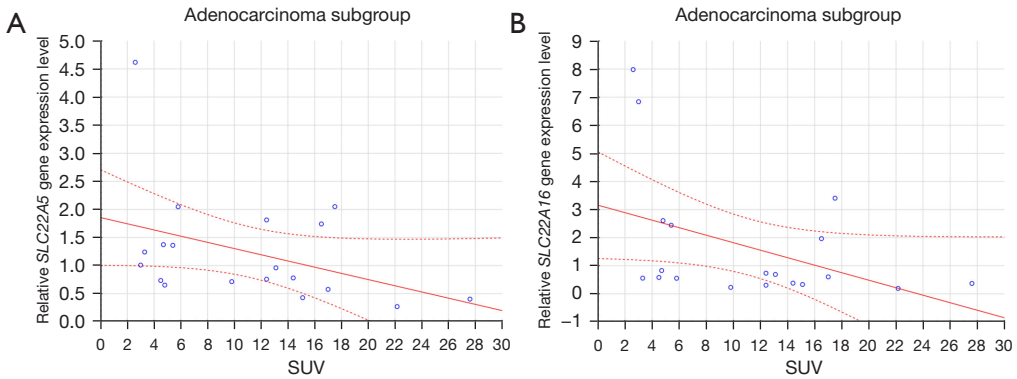

Figure 2 The correlation between relative *SLC22A5* and *SLC22A16* gene expression level and SUV parameter of LUAD patients. Line represents a linear model fit whereas the dashed line indicates 95% confidence interval, raw data are presented as blue dots. LUAD, lung adenocarcinoma; SUV, standardized uptake value.

correlation for *SLC22A5* and *SLC22A16* genes expression level in the LUAD subtype with standardized uptake value (SUV) (r=–0.40, P=0.02, Q=0.03, *Figure 2A* and r=–0.43, P=0.04, Q=0.04, *Figure 2B* respectively). The tumors with higher SUV had lower expression levels of both aforementioned genes. Metabolically stimulated tumors seem to have reduced expression of genes encoding carnitine transporters and, consequently, altered metabolism of this compound.

However, in all patients and separately for men/women and smokers/non-smokers, there was no significant correlation between the expression levels of selected genes and pack-years, body mass index (BMI), hemoglobin and age (all P values >0.05). No dependences were also found for individual cancer stages and TNM (all P values >0.05).

The next step of the analysis included assessing the differences in the level of selected genes and the occurrence of cancer in the family or previously in the patients. No differences were observed in the expression levels of selected genes and the occurrence of cancer in the family of patients diagnosed with LC or in the patient with a previous history of cancer, even after division to subgroups according to sex and tobacco smoking status (all P values >0.05).

When we compare the expression levels of selected genes from the *SLC* family depending on the type of cancer, significant differences are observed between the AC and SCC subtypes for the *SLC6A14* gene (P=0.003, Q=0.01, *Figure 3A*). Furthermore, after taking into account patients' sex and tobacco smoking, these differences remain significant in subgroup of men and active smokers (P=0.007, Q=0.01, *Figure 3B* and P=0.009, Q=0.01, *Figure 3C*), however were lost in women and non-smokers (P=0.46 and P=0.55).

Additionally, in the study group, strong and moderately strong positive interrelations were observed between the SLC genes themselves, namely between the *SLC22A5* gene and *SLC6A14* (r=0.56, P and Q<0.001) and *SLC22A16* (r=0.74, P and Q<0.001), also between the *SLC6A14* and *SLC22A16* (r=0.32, P and Q<0.001). After division into subtypes, these associations deepened in the case of SCC (all P and Q<0.001, r=0.90, r=0.72, r=0.54 respectively), but disappeared in the case of AC. This may indicate a different mechanism of molecular changes in both subtypes of LC. The squamous subtype appears to be more associated with altered carnitine transporters and thus, different metabolism.

External databases

The mRNA expression of chosen *SLC* gene family members was identified in various cancer types and normal adjacent tissue pairs using the TIMER database. The significant downregulation compared to normal adjacent tissue was observed for *SLC22A5* in LUSC and for *SLC6A14* in both LUAD and LUSC subtypes (*Figure 4*).

The correlation between patient survival and *SLC* genes family expression was analyzed by using KM plotter, with the follow-up threshold of 60 months (5 years). The analysis evaluating the effect of the *SLC22A5*, *SLC22A16* and *SLC6A14* gene expression at the time of diagnosis on the survival time of LC patients revealed that lower expression correlated with a shorter 5 years OS (all P values

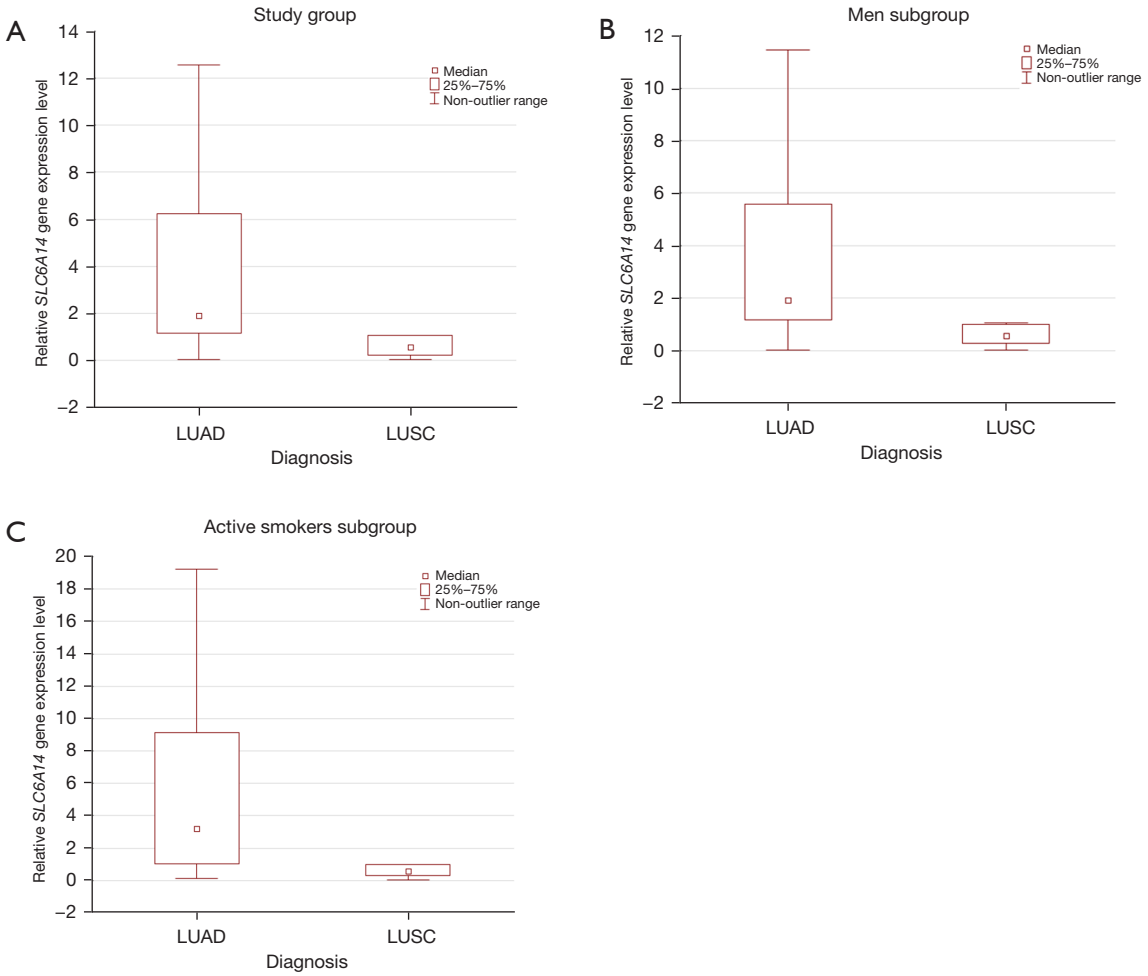

Figure 3 The differences in relative *SLC6A14* gene expression level in LUAD and LUSC patients (A), in the subgroup of men (B) and in active smokers (C). LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

<0.01, *Figure 5A*). After division to subtypes, the association remained significant for *SLC22A16* in LUAD, but was lost for *SLC6A14* (*Figure 5B*). The contrary was observed for *SLC22A5* gene in LUAD, where higher expression was connected to worse prognosis. In LUSC subtype only significant finding was connection between *SLC6A14* higher gene expression level and longer OS in patients (P=0.01, *Figure 5C*).

Discussion

It is known that SLCs' transport a diverse array of substrates in various essential physiological processes, including ion transport, nutrient uptake, waste removal or drug absorption (43,44). Moreover, this gene family is often dysregulated in human diseases, especially cancer, suggesting its potential therapeutic function (45-47).

The modulation of SLC transporter activity presents a promising avenue for cancer therapy, offering opportunities to disrupt the metabolic dependencies of cancer cells, enhance the efficacy of existing treatments, and circumvent drug resistance mechanisms. Thus, the detailed understanding of SLC transporter dysregulation in cancer not only enriches our comprehension of tumor biology but also opens new horizons for targeted therapeutic interventions (16,30,38,39).

Some data indicate that 70% of *SLC* family genes were differentially expressed between LUAD tissues and adjacent normal tissues (48). Moreover, in LUAD aberrant expression of *SLC* family genes has been reported to be

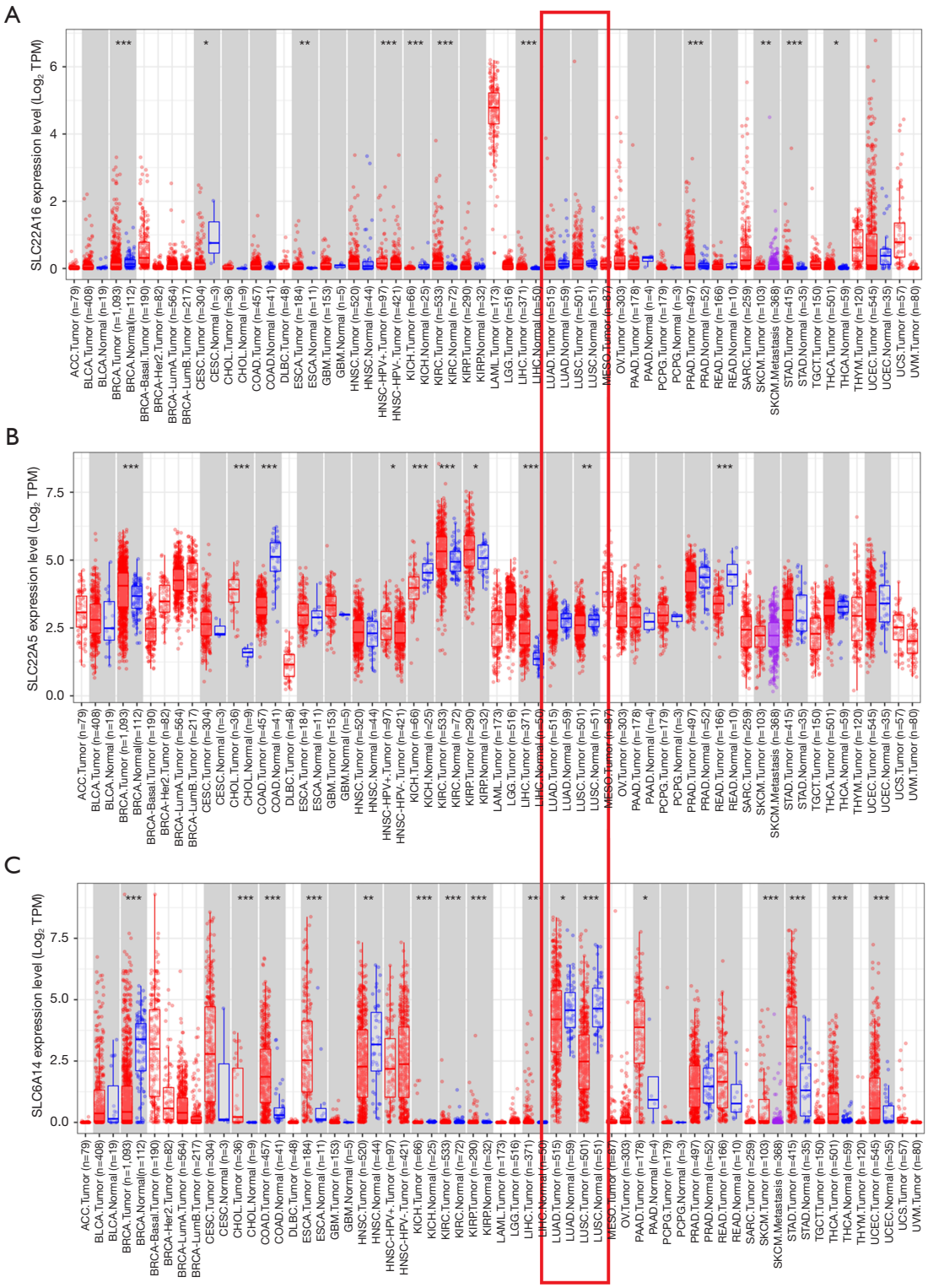

Figure 4 The mRNA expression of *SLC22A16*, *SLC22A5* and *SLC6A14* family genes in various cancers and adjacent normal tissues from the TIMER database. Data are presented as red (tumor) and blue (normal tissue) box-plots and raw data are symbolized by dots. Significant differences are shown with asterisks: *, P<0.05; **, P<0.01; ***, P<0.001. LUAD and LUSC data are highlighted by red border. TIMER, Tumor Immune Estimation Resource; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; TPM, transcript per million.

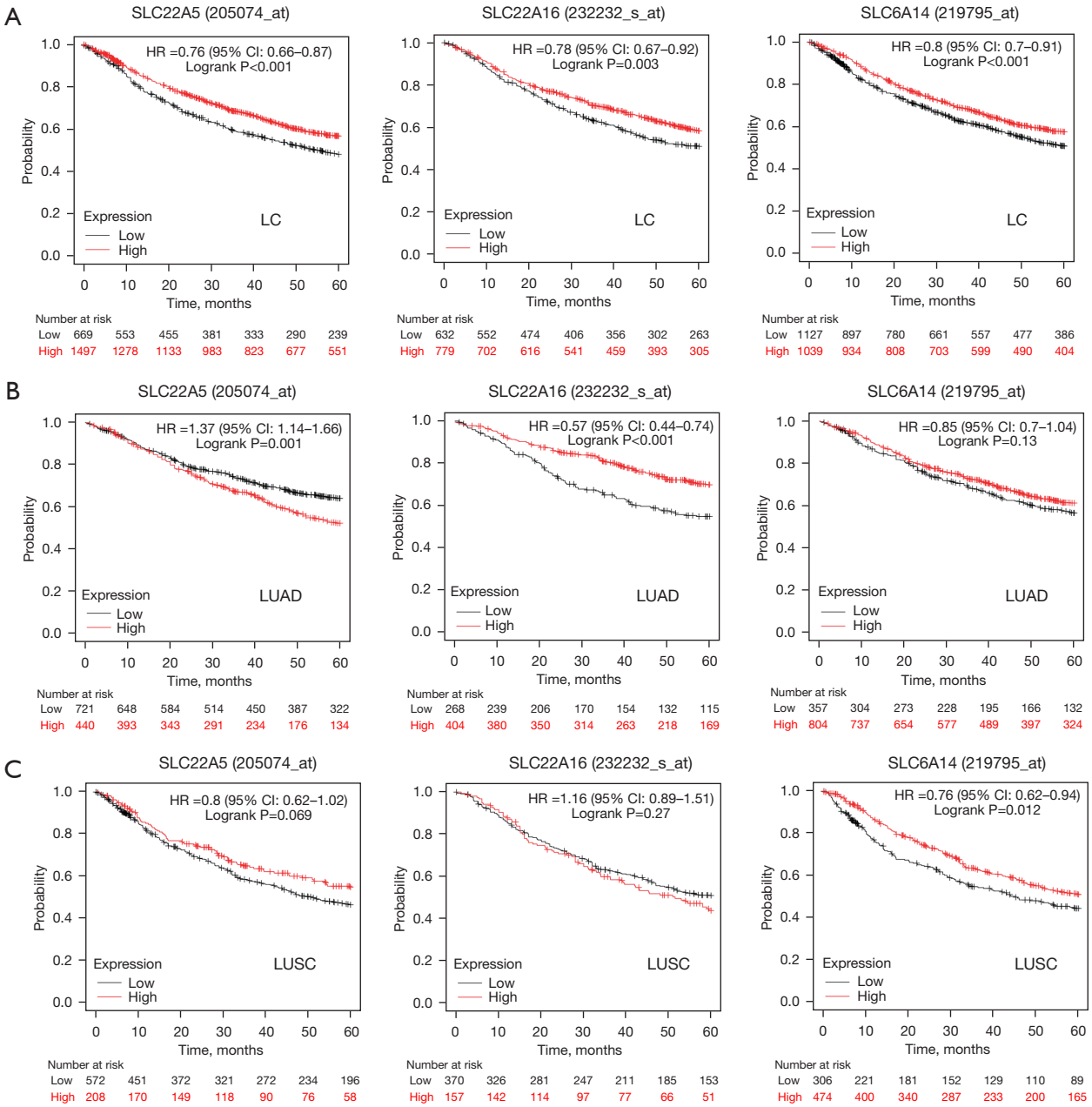

Figure 5 The survival curves for chosen SLC family genes expression (low *vs.* high expression level) with patient OS (60 months) in LC (A), LUAD (B), and LUSC (C) for *SLC22A5*, *SLC22A16* and *SLC6A14* genes respectively. The survival curves were retrieved from the Kaplan-Meier plotter, as described in Methods section. OS, overall survival; LC, lung cancer; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; HR, hazard ratio; CI, confidence interval.

associated with cellular proliferation and survival, and they may be useful diagnostic and prognostic biomarkers. Some of these genes are also associated with patients' prognosis and their expression was correlated with hazards ratio (HRs). Furthermore, *SLC* genes family-based signature accurately predicts survival outcomes in LUAD patients (49-52).

Nevertheless, the expression profiles and clinical value of *SLC* family members in LC remain largely unexplored.

From the above results, it is clear that the chosen *SLC* gene family members are connected to the LC occurrence,

progression and survival and the difference is also observed between LUAD and LUSC patients.

The *SLC22A5* gene is overexpressed in cancers, which are located in the endometrium, ovary, pancreas, kidney and in glioblastoma multiforme. It was also noted that increased expression level of the *SLC22A5* gene is associated with worse patient survival rate. On the other hand, downregulation of *SLC22A5* gene expression level leads to tumor cell necrosis (30,53-55). Through undertaken analysis the downregulation of this gene was observed in LUSC patients, what is more this gene expression was connected to patients' survival rates, in particular higher *SLC22A5* gene expression was associated with longer OS in LC, but on the contrary in LUAD—with shorter 5-years survival. Moreover, high *SLC22A5* gene expression negatively correlated with SUV parameter in LUAD patients, which may indicate that metabolic stimulation of tumors changes the carnitine and/or FAO uptake.

Overexpression of the *SLC22A16* gene was observed in acute myeloid leukemia and in intestinal cancer. It has been proven that reduction of the *SLC22A16* gene expression level in tumors shortens the patients' lifespan. The SLC22A16 protein may also be an important therapeutic target due to the fact that it is involved in the transport of anti-cancer drugs. The increased expression level of *SLC22A16* gene affects the cisplatin treatment in LC cases (cisplatin is a substrate recognized by this transporter), in particular, the overexpression of *SLC22A16* gene is correlated with increased cisplatin uptake and intracellular concentration of this drug (30,33,34,56-60). The TIMER database did not reveal any differences in *SLC22A16* gene expression level between cancer and normal adjacent tissues, however through KM Plotter analysis it was discovered that higher gene expression level correlated with longer OS, especially for LUAD patients. It stays with the contrary to literature findings and is particularly interesting. What is more, the high *SLC22A16* gene expression negatively correlated with SUV parameter in LUAD patients, metabolically stimulated tumors seem to have reduced expression of genes encoding carnitine transporters and, consequently, altered metabolism of this compound. No other associations were found for this gene and patients' clinicopathological features.

A high expression level of *SLC6A14* gene was observed in the salivary glands, skin, breast and lung tissue. Studies have shown that overexpression of the *SLC6A14* gene has been observed in many types of cancers such as: colorectal, estrogen receptor-positive (ER^+) breast, pancreatic and cervical cancers (38,61). Overexpression of SLC6A14 protein supplies carnitine as an essential cofactor to cancer cells where FAO is responsible for energy production (38,61-64). This analysis surprisingly revealed significant downregulation of *SLC6A14* gene expression level in LUAD and LUSC compared to normal adjacent tissues. There were also differences between individual LC subtypes, in particular the LUSC subtype presented significant lower *SLC6A14* mRNA level as compared to LUAD. The tobacco smoking also had influence on *SLC6A14* gene expression level in LUSC subtype, where it causes downregulation. What is more, its prognostic feature was revealed in overall LC and LUSC subtype, where higher gene expression correlated with longer 5-year OS of patients.

It is worth mentioning that other genes from the SLC family have also been tested for their clinical and prognostic utility, e.g., *SLC2A1*, encoding the GLUT1 protein. In the review work undertaken by Pezutto and others (65), it was shown that it can act as a prognostic factor that affects tumor aggressiveness, interact with the immune system and its higher expression is present in metastatic sites. More importantly, this GLUT isoform is the only one that correlates positively with SUV index, contrary to *SLC22A5* and *SLC22A16*, obtained through this research. To date, few studies have evaluated the impact of patient-related factors, SUV levels, and gene expression levels. We hypothesize that higher SUV values indicate that these tumors are more dependent on glucose than on fatty acids, which may explain the observed negative correlation with carnitine transporter genes expression. However, this analysis cannot fully elucidate the underlying mechanisms, as this is the first report of such an observation. Nevertheless, this finding could serve as a basis for further investigations, including functional assays and protein level determination.

In recent years, many researchers have turned their attention to non-invasive tests in patients' body fluids, the so-called liquid biopsy. It is impossible not to mention this non-invasive method when searching for new biomarkers and potential prognostic indicators of the cancer process. Liquid biopsy shows potential in circumventing tumor heterogeneity, identifying patients who may respond to targeted therapy, dynamically monitoring treatment effects and revealing the mechanism of drug resistance. Currently, circulating tumor cells (CTCs), circulating tumor DNA (ctDNA) and exosomes are the most commonly detected biomarkers through liquid biopsies (66,67). Future studies would also benefit from evaluating whether changes of SLC genes observed in the tumor tissue are reflected in body

fluids, by performing comparative studies of tissues and liquid biopsies.

Scientific sources report that in recent years researchers have obtained promising results for utilizing liquid biopsies in LC patients. The team led by Rossi and co-authors (68) constructed a test based on ctDNA that detected the *EML4-ALK* gene fusion. This assay not only successfully detected rearrangement, but was also evaluated as a predictive marker, with shorter progression-free survival (PFS) in patients positive for EML4-ALK. This is a very important finding due to the ease of performing the test in body fluids. Such studies encourage undertaking activities and searching for other markers that can be determined by liquid biopsy and thus become a diagnostic and prognostic tool in NSCLC.

The findings from our study illuminate the variance in expression levels of genes encoding carnitine transporters, hinting at a potentially altered carnitine metabolism in LC patients. Notably, this variance is not uniform but rather exhibits specificity across LC subtypes, with marked distinctions between SCC and AC. Furthermore, the correlation between gene expression levels and OS of patients underlines the prognostic significance of *SLC* genes within these cancer subtypes. Our study has some limitations; the obtained results are limited to the Polish population with a restricted sample size, some analyses were performed on publicly available data from TCGA dataset and thus should be verified in prospective, clinical trials, which will take into account the mechanisms underlying observed changes among selected *SLC* genes.

Conclusions

Our research contributes to the burgeoning body of evidence that suggests the *SLC* gene family plays a crucial role in LC pathogenesis and progression. The differential expression of these genes across LC subtypes (LUAD and LUSC) opens new pathways for targeted molecular interventions. This insight lays a foundational stone for the design of innovative clinical trials aimed at exploring targeted therapies that address these molecular discrepancies. Given the complexity and heterogeneity of LC, further investigations into the *SLC* gene family's role are imperative. Future research should focus on unraveling the intricate mechanisms through which these transporters influence cancer metabolism, resistance to therapy, and patient outcomes. It can move closer to the development of precision medicine strategies that offer improved patient prognosis and survival in LC.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-448/rc

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-24-448/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Medical University of Lodz (Nos. KE/952/22 and RNN/87/16/KE) and informed consent was obtained from all individual participants.

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