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Surgical resection of lung cancer inhibits mRNA expression of GOT2 gene encoding kynurenine aminotransferase in leukocytes

Tomasz Karol Prystupa[1,A-D] ✉, Dariusz Sagan[1,A,E], Janusz Kocki[2,A], Tomasz Kocki[3,A], Rafał Szymanowski[2,C], Jacek Bogucki[2,C]

[1] *Thoracic Surgery Department, Medical University, Lublin, Poland*
[2] *Department of Clinical Genetics, Medical University, Lublin, Poland*
[3] *Department of Clinical Pharmacology, Medical University, Lublin, Poland*
A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of the article

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Abstract

Introduction and Objective. Lung cancer is the most common malignant tumour. More than 80% of all diagnosed cases are non-small cell carcinoma which can be effectively treated by radical resection. Despite significant progress in the field of diagnostic and therapeutic methods, the results of lung cancer treatment are still unsatisfactory. Lung cancer is detected relatively late, which leads to an unfavourable prognosis. Kynurenine aminotransferases are an important element of the kynurenine pathway of tryptophan metabolism, which has recently aroused great interest from the aspect of possible use as a target point of personalized therapies in malignant tumours.The aim of the study was to analyze the expression of the selected gene of kynurenine aminotransferases GOT 2 at the mRNA level in peripheral blood leukocytes of patients with lung cancer.

Materials and method. The mRNA expression of the GOT 2 gene was tested on blood samples from 50 patients treated surgically for non-small cell lung cancer.The control group consisted of 15 healthy individuals.The determination of mRNA expression of the GOT 2 gene was performed using the real-time PCR method.The GAPDH gene was used as the endogenous reference level.

Results. The mRNA expression of the GOT2 gene on the 6th day after surgery was statistically significantly lower than before surgery (p = 0,05). In the study group, the average LogRQ mRNA expression of the GOT2 gene before the procedure was 0.192082±0.292174 in woman. This was statistically significantly higher than in men whose average LogRQ mRNA expression of the GOT2 gene before the procedure was 0.004210±0.235065 (p=0.0183).

Conclusions. Surgical resection of lung cancer results in inhibition of GOT2 mRNA expression in leukocytes. Further studies are expected to show whether it may be used as a target point for personalized therapies in lung cancer.

Key words

mRNA, Lung cancer, GOT2, Kynurenine aminotransferases

INTRODUCTION

Lung cancer is currently the most common primary malignant tumour in Poland and worldwide, and is regarded as the leading cause of cancer-related mortality in developing countries [1]. The effects of carcinogenic substances contained in tobacco smoke may promote growth, metaplasia or neoplastic transformation [2].

Small cell lung cancer (SCL) displays other biological and clinical features different from other types. The differences are due to a higher degree of proliferation, a shorter tumour doubling time, and a tendency to metastasize early [1]. SCL is chemo-sensitive and relatively radio-sensitive. In the past, it was justified to use a practical division into small cell and non-small cell carcinomas for which early thoracic surgery is an effective treatment [1]. Lung cancer is a malignant tumour with clinical symptoms at a late stage of progression which, in its early development, may be asymptomatic [2, 3]. It may be discovered incidentally during a routine chest radiography without the typical symptoms appearing previously [3].

Methods of surgical treatment of lung cancer. Currently, radical surgical resection of the tumour at an early stage is accepted as the gold standard for effective treatment of non-small cell lung cancer (NSCLC) [4]. Surgical treatment is important in patients with I – IIIA clinical stages [5]. Careful selection of patients in terms of interdisciplinary health assessment by pulmonologists, thoracic surgeons and oncologists is important prior a planned thoracic surgery [6]. Over the years, thoracic surgeries have undergone some significant changes with regard to the way of opening the chest, access to the pleural cavity, and resection of the lung parenchyma. Minimally invasive video-assisted thoracoscopic surgery (VATS) techniques are becoming the method of choice in thoracic surgery worldwide. Anatomical procedures to remove the lung parenchyma, such as segmentectomy, lobectomy, bilobectomy, and even pulmonectomy, are performed only with two or three port incisions. Currently, the technique of performing the above-procedures using only one Uniportal VATS utility incision,

✉ Address for correspondence: Tomasz Karol Prystupa, Thoracic Surgery Department, Medical University, Lublin, Poland
E-mail: Tprym@wp.pl

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without excessive expansion of the intercostal spaces, is being developed [6].

Thoracic surgery is also applicable in cases of non-small cell lung cancer in the remaining lung after previous contralateral pneumonectomy. In such patients, limited wedge resection of the nodule is recommended after evaluation of current imaging studies. Although such thoracic surgeries are rare, they might provide a satisfactory post-operative outcome [7, 8].

Prognosis and quality of life of patients after lung cancer surgery. Lung cancer is one of the most common malignancies with a very high mortality rate in the world's population. Its early detection and surgical resection are an effective method of treating non-small cell carcinoma in stage I and II, and in some patients in stage IIIA, depending on the pre-operative assessment [8]. Radical resection of lung cancer allows the prolongation of life and improves its quality [9]. The five-year survival rate of patients, however, is low due to the diagnosis of the disease at an advanced stage. Late diagnosis of lung cancer reduces the five-year survival rate among patients undergoing thoracic surgery [9].

Kynurenine aminotransferase GOT2. Aspartate transaminase (ASAT) was first discovered in Escherichia coli bacteria as the kynurenine transamination activity of mitochondrial ASAT. [10]. Otherwise, KAT IV is recognized as an enzyme that plays an important role in the production of kynurenic acid in the mouse, rat and human brains. As mitochondrial ASAT or GOT 2 enzymes, they participate in the catalyzing reaction of the reversible transamination of oxaloacetate into aspartate, with the participation of the conversion of glutamate into alpha-ketoglutarate [11]. Other functions performed by KAT IV include the synthesis of glutamate as a neurotransmitter in the central nervous system [12], as well as the regeneration of intramitochondrial glutamate from intermediate metabolites derived from the course of the tricarboxylic acid cycle [13].

GOT2 is well-known as a member of the malate-asparate shuttle, a cytosolic-mitochondrial pathway that transfers reducing equivalents into the mitochondria to support oxidative phosphorylation [14]. GOT2 also participates in several critical metabolic functions, for example: nucleotide synthesis, redox homeostasis, fueling the tricarboxylic acid (TCA) cycle, fatty acid transport, nitrogen balance, and sulfur catabolism [14].

In addition, The deregulated cell growth and division in cancer cells imposes significant requirements for RNA and DNA. Some researchers consider that the most well-studied product of the GOT2-catalyzed reaction is the amino acid aspartate, which is a rate-limiting metabolite for cancer cells due to its contribution of both carbon and nitrogen to nucleotide synthesis [15, 16, 17, 18]. GOT2 is at the nexus of several critical metabolic pathways in homeostatic cellular and dysregulated cancer metabolism in pancreas and colorectal cancer [19]. In other new examinations, GOT2 is a plasma membrane-bound fatty acid-binding protein, which has provided an alternative protein name – FABPpm. In this role, GOT2/FABPpm is responsible for the import of free fatty acids under conditions of cellular stress, organ injury, or fatty acid accumulation, potentially influencing fatty acid oxidation [20, 21] GOT2 as a plasma-membrane bound fatty acid transporter in several tissues, this capacity has only recently been examined in cancer. One provocative study confirmed that GOT2 as a fatty acid-binding protein in pancreatic cancer [22]. The tissue of origin plays a critical role in the tumour-promoting or tumour-restraining function of GOT2 in cancer metabolism. For example, in hepatocellular cancer, down-regulation of GOT2 is associated with a more aggressive disease and worse outcomes. Indeed, over-expressing GOT2 in hepatocellular cancer cell lines restrains tumour growth. One potential explanation is that loss of GOT2 increases pools of the GOT2 substrate glutamate, increasing its incorporation in glutathione [23]. In colorectal cancer, GOT2 is important in nitrogen balance through the production of amino acids and the urea cycle via a HIF1a-SOX12-GOT2 axis that drives asparagine production from aspartate. Inhibition of this pathway impairs cell growth [24]. The latest reports indicate other biochemical aspects in which KAT IV is involved, but this requires further research.

OBJECTIVE

Preliminary research results suggest that the kynurenine pathway of tryptophan metabolism plays an important role in the pathogenesis and development of neoplastic diseases. This is very important in the effectiveness of diagnosis and improvement of better surgical treatment in patients with lung cancer. There exist only a few studies dealing with the subject of the kynurenine acid metabolic pathway in lung cancer which, otherwise, seems to be very promising in clinical terms. Therefore, the main aim of the presented study was to analyze the mRNA expression of the gene encoding kynurenine aminotransferase GOT 2 in peripheral blood leukocytes of lung cancer patients, both before and after surgical procedure, on the 3rd and 6th day. An additional aim was to answer the question of whether the gender of the patients differentiates the level of GOT2 gene expression.

MATERIALS AND METHOD

Sixty-five patients, 43 men and 22 women, were enrolled in the study. The age of the participants ranged from 24 – 82 years; on average – 60 years. The patients participating in the study were divided into two groups, a study group and a control group. The study group consisted of 50 participants treated surgically for lung cancer at the SPSK4 Department of Thoracic Surgery at the Medical University of Lublin, south-east Poland, in the period May 2019 – August 2020. The study group consisted of 18 women and 32 men. The age of the patients ranged from 56 to 82 years, with an average of 67.7 years. All patients came from the Lublin Province and had a confirmed tobacco smoking history. Among the patients, 20 had a confirmed presence of lung cancer in the family – 40% of the respondents. The study group included patients with confirmed post-operatively histopathologically non-small cell lung cancer.

Thoracic surgeries were performed under general endotracheal anesthesia with double-lumen intubation and separate lung ventilation. The procedures were performed from the anterolateral thoracotomy or anterior thoracotomy. In some cases, the VATS method was used, supported by a video track. The scope of the resection was dependent on the intraoperative and histopathological diagnosis, stage of

advancement, location of the primary lesion, and anatomical conditions in the operating field, taking into account the pre-operative spirometry and cardio-circulatory tests. Most often, the patient's left lung was operated on – 30 patients, and on the right lung – 20 cases. The control group consisted of 15 healthy subjects – 11 men and 4 women, aged 22 – 58, with an average age of 35. In the control group, venous blood serum, which was collected from the basilic vein in the morning, was tested. The healthy people were fasting.

Before collecting blood and after discussing the purpose of the study, all subjects gave written consent confirming their agreement to participate in the study. The results of blood tests in the control group were within the normal range of laboratory parameters.

Test method. By Resolution No. KE-0254/198/2010, the Bioethics Committee of Medical University of Lublin expressed a positive opinion on the research project and consented to it being carried out. Written consent was provided by all patients after being fully informed about the nature of the study, collection of material, possible side-effects, and the potential scientific, medical and social value of the study. Anonymity of medical data and research results was assured in accordance with the currently applicable rules of the Data Protection Law regarding scientific research.

The material collected for laboratory tests from the study group consisted of three blood samples: venous blood collected on the 1st day before thoracic surgery, on the 3rd day after the procedure, and on the 6th day after the procedure. The final histopathological result of the changes in the lung, confirmed by the Department of Pathomorphology of Medical University of Lublin, was awaited. In the control group, venous blood was also collected for testing. A 4.9 ml tube-syringe in the Sarstedt system was used for blood collection. Fasting blood was collected from the basilic vein using a Monovette needle directly into the serum activator tube-syringe of the Sarstedt closed blood collection system. The venous blood collected in this way was centrifuged at 4,000 rpm for 10 minutes, followed by 1 ml of blood serum centrifuged as supernatant, being pipetted into an Eppendorf tube. The serum samples obtained were left in Eppendorf tubes and were immediately frozen at -70^{0}C and stored in a deep-freeze freezer. Reagents used in molecular studies to determine the expression of kynurenine aminotransferase genes (Applied Biosystems) using the PCR 7300 Real Time PCR System (Applied Biosystems). Other standard chemicals were purchased from Polish Chemical Reagents SA (Gliwice, Poland). The research was carried out at the Department of Experimental and Clinical Pharmacology of Medical University of Lublin. The procedure of RNA isolation from biological material, venous blood, was carried out according to Chomczyński and Sacchi's method. Leukocytes homogenization was performed manually in 1.5 ml Eppendorf centrifuge tubes with the use of Eppendorf Research Plus automatic pipettes from Eppendorf. The samples were not centrifuged. The suspension obtained as a result of homogenization was subjected to subsequent treatments. 0.2 ml of chloroform was added to each tube.

A significant step was the removal of protein impurities and the separation of DNA from RNA. The samples were shaken by hand for 10–15 seconds, and left at room temperature for 15 minutes. After this time, the tubes were placed in a 5415R centrifuge, which was then cooled to 4 °C and centrifuged at 13,600 rpm. for 15 minutes. In this way, the contents of the tubes were separated into an aqueous phase containing RNA, an interphase, and finally organic phase. The last two phases contained DNA as well as denatured proteins. The aqueous phase was collected and transferred to new 1.5 ml centrifuge tubes, followed by the addition of 0.5 ml isopropyl alcohol to precipitate the RNA from the presented solution. The contents of the tubes were gently mixed by tilting them at 180° several time, and left at room temperature for 20 minutes. The tubes were then placed in a 5415R Centrifuge, cooled to 4 °C and centrifuged at 13,600 rpm for 20 minutes. The RNA-containing pellet obtained in this way was dried by draining off the supernatant and flooded with 0.25 ml of 80% aqueous ethanol at -20 °C. The reverse transcription reaction procedure was performed according to the instructions provided by the manufacturer of the reagents (Applied Biosystems).

The first stage of the procedure was to check two important features, i.e. purity and concentration of RNA obtained as a result of isolation with the TRI reagent. For this purpose, the RNA sample was dried and then dissolved in 12 µl of ultrapure water (UPW), and the tube placed on ice. Spectrophotometric analysis was then performed on a NanoDrop 2000c Spectrophotometer (ThermoFisher). For this purpose, 2 µl of the tested suspension were placed in the device and the result read, calculated on the basis of absorbance values at 260 and 280 nm. Sample purity was expressed as an absorbance ratio of 260/280 nm, and if the result was in the range of 1.8–2, the sample was introduced to the significantly most important reverse transcription reaction. Further procedure involved mixing the reagents contained in the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) (Tab. 1). RNA diluted with ultrapure water was added to the prepared mixture to a concentration of 1 µg/10 µl.

Table 1. Quantitative and qualitative composition of the reaction mixture

Lp.	Reagent	1 sample volume
1	ultrapure water (up to 20 µl)	3.2 µl
2	10x RTbuffer	2 µl
3	10xdNTPs (100mM)	0.8 µl
4	10xRT Random Primer	2 µl
5	RNazin 20U/µl	1 µl
6	Reverse transcriptase 50U/µl	1 µl

The mixture in a volume of 20 µl was prepared in very tightly closed polypropylene conical tubes of 200 µl each (Sarstedt). The tubes were then placed in a Veriti thermal cycler (Applied Biosystems). The conditions under which the reaction took place are presented in Table 2.

Table 2. Reverse transcription in reaction conditions

Parameters	Stage I	Stage II	Stage III	Stage IV	Stage V
temperature	25 °C	37 °C	37 °C	85 °C	4 °C
Time	10 min.	60 min.	60 min.	5 min.	∞

After the reaction, 20 µl of cDNA solution was obtained which was then used to study the expression of selected genes. The real-time PCR (rtPCR – real time Polymerase Chain Reaction) method was then used to determine the expression

of the GOT 2 gene. The gene encoding glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was selected as an endogenous sample. TaqMan probes and MasterMix (Applied Biosystems) were used and reactions performed in a MicroAmp Enduraplate mul-well plate (Applied Biosystems). Probe, cDNA, MasterMix and water were then added to each well. The MasterMix buffer contained i.a. DNA polymerase, uracil-DNA glycosiclase, deoxynucleotide triphosphates (dNTPs), deoxyuridine triphosphate (dUTP), and a buffer. The exact quantitative and qualitative composition of the reaction mixture is presented in Table 3.

Table 3. Quantitative and qualitative composition of reaction mixture

Lp.	Reagent	Volume
1	probe TaqMan	1.25 µl
2	buffer MasterMix	12.5 µl
3	cDNA	1 µl
4	Ultrapure water	10.25 µl

The multi-well plate with all components of the reaction mixture was placed in a StepOne Plus apparatus (Apllied Biosystems). The conditions under which the reaction was observed are presented in Table 4.

Table 4. Reaction conditions PCR

Parameters	Stage I	Stage II	Stage III*	Stage IV*
Temperature	50 °C	95 °C	95 °C	60 °C
Time	2 min.	10 min.	15 sec.	1 min.

* stages III i IV repeated 40 times

The TaqMan probe used for the reaction is an oligonucleotide with a specific sequence, labeled with a fluorochrome at the 5' end (reporter), and a quencher at the 3' end. When both ends are close to each other, the fluorescent signal of the reporter is absorbed by the quencher. If a specific product appears as a result of the PCR reaction, the probe hybridizes to the DNA strand. As the reaction product elongates, the probe is degraded by the polymerase. Taq is an enzyme with 5'-exonuclease activity. Ultimately, this leads to an increase in the distance between the reporter and the screensaver. The intensity of the fluorescence emitted in this way is measured by the device after each reaction cycle. The integrated DNA polymerase chain reaction apparatus PCR 7300 Real-Time PCR System (Applied Biosystems) was used in molecular studies. The research was carried out in the Independent Laboratory of Clinical Genetics of the Medical University of Lublin.

Statistical analysis. Statistical analyses were performed using Statistica 13.3 software (StatSoft Inc) and Excel for charts (Microsoft Corp.). Descriptive statistics of analyzed variables are given as mean (M), median (Me), minimum (Min.), maximum (Max.), standard deviation (SD). The number of groups in statistical analyses, Tables and Graphs, is marked with the symbol 'N'. The data distribution of the analyzed variables differed statistically significantly from the normal distribution; therefore non-parametric statistical tests were used in the further analysis. For comparisons between two independent groups, the Mann-Whitney U test was used. Freedman's ANOVA test was used to analyze between the three dependent groups. The level of statistical significance was set at 0.05

RESULTS

In the study group of 50 patients with lung cancer – 32 men and 18 women – the analysis was performed of the expression of mRNA of the *GOT2* gene in venous blood leukocytes before surgery, on the 3rd day after surgery, and on the 6th day after surgery. The results were compared with the control group – 11 healthy men and 4 healthy women. The constitutive Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) gene was used as the endogenous reference level. Appropriate descriptive statistical methods were used in the study along with the Mann-Whitney U test. The logarithmic value of the gene mRNA expression result was used for statistical analysis of *GOT2* gene mRNA expression. Descriptive statistic of LogRQ *GOT2* mRNA expression of the study groups are presented in Table 5.

Table 5. Descriptive statistic of LogRQ *GOT2* mRNA expression of the study groups

Variable	N	M	Me	Min.	Max.	SD
LogRQ *GOT2* before surgery	36	0.0824	0.0675	-0.5506	0.6992	0.273
LogRQ *GOT2* on the 3rd day after surgery	47	-0.0339	0.0014	-0.6906	0.5205	0.2994
LogRQ *GOT2* on the 6th day after surgery	46	0.000039	0.0506	-0.7809	1.9138	0.4345

The results of the analysis of the significance of differences between study groups, performed using the ANOVA Friedman test are shown in Table 7.

3rd day after surgery and 6th day after surgery in the study group.

The result of the statistical analysis of the LogRQ mRNA expression of the *GOT2* gene in the study group showed statistically significant differencess (p=0.011) between the analyzed groups (Fig. 1). Analysis of multiple comparisons showed that expression level of LogRQ mRNA expression of

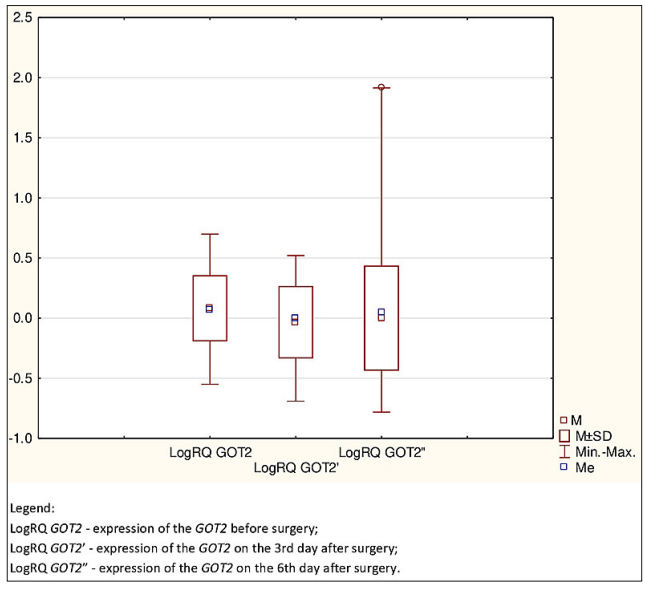

Figure 1. mRNA expression of GOT2 gene in study groups before surgery

Table 6. ANOVA Friedman test results

Variable	ANOVA Friedman and conformity factor of Kendall Chi². ANOVA(N = 34, df = 2) = 8,8823 p = 0,011 conformity factor= 0,13062 r mean. rang =,10428			
	Mean (Range)	Summary (Rang)	Mean	SD
LogRQ *GOT2* before surgery	2.4117	82	0.0666	0.2727
LogRQ *GOT2* on 3rd day after surgery	1.8529	63	-0.0565	0.31892
LogRQ *GOT2* on 6th day after surgery	1.7352	59	-0.0744	0.3461
Variable	Absolute differences between average range re approximate if > 0.580625387073338 at the significant level 0.05*			
	LogRQ GOT2 before surgery	LogRQ GOT2 on 3rd day after surgery	LogRQ GOT2 on 6th day after surgery '	
LogRQ *GOT2* before surgery	---	0.5588	**0.6764***	
LogRQ *GOT2* on the 3rd day after surgery	0.5588	---	0.1176	
LogRQ *GOT2* on the 6th day after surgery	**0.6764***	0.1176	---	

the GOT 2 gene on the 6th day after surgery was statistically significantly (0.6764, significant level – 0.05), which was lower than LogRQ mRNA expression of the *GOT2* gene before surgery

The next step of the analysis was to determine whether the level of LogRQ mRNA of the *GOT2* gene expression differed between male and female patients. The first step was to calculate descriptive statistics for the male and female patients.

Table 7. Discriptive statistic of LogRQ GOT2 mRNA expression in males. Gene expression variables in men before surgery on 3rd and 6th day after surgery

Variables	Gender = Men. Descriptive statistics					
	N	M	Me	Min.	Max.	SD
LogRQ *GOT2* before surgery	21	0.0042	0.0152	-0.4388	0.5487	0.235
LogRQ *GOT2* on 3rd day after surgery	29	-0.0394	-0.0015	-0.6906	0.5129	0.2829
LogRQ *GOT2* on the 6th day after surgery	32	-0.0172	0.0077	-0.7809	1.9138	0.5163

Table 8. Discriptive statistic of LogRQ GOT2 mRNA expression in women. Gene expression variables in women before surgery on 3rd and 6th day after surgery

Variables	Gender = Woman Descriptive statistics					
	N	M	Me	Min.	Max.	SD
LogRQ *GOT2* before Surgery	15	0.192	0.2478	-0.5506	0.6992	0.2921
LogRQ *GOT2* on 3rd day after surgery	18	-0.025	0.0019	-0.5233	0.5205	0.3325
LogRQ *GOT2* on 6th day after surgery	16	0.0326	0.0935	-0.3635	0.3326	0.2222

The results of the analysis of the significance of differences in the level of mRNA expression of the examined gene *GOT2* for men and women using the Mann-Whitney U test are presented in Table 9.

Among all variables analyzed with the Mann-Whitney U test, statistically significant differences were found with regard to the preoperative mRNA expression of the *GOT2* before surgery gene (U=84; Z=2,358; p=0.0183) between group of male and female patients. The mRNA expression of the GOT 2 before surgery gene was statisticaly higher in female patients. The remaining analyzed variables did not show a statistically significant relationship with mRNA expression of other genes depending on sex and time of material collection (Fig. 2).

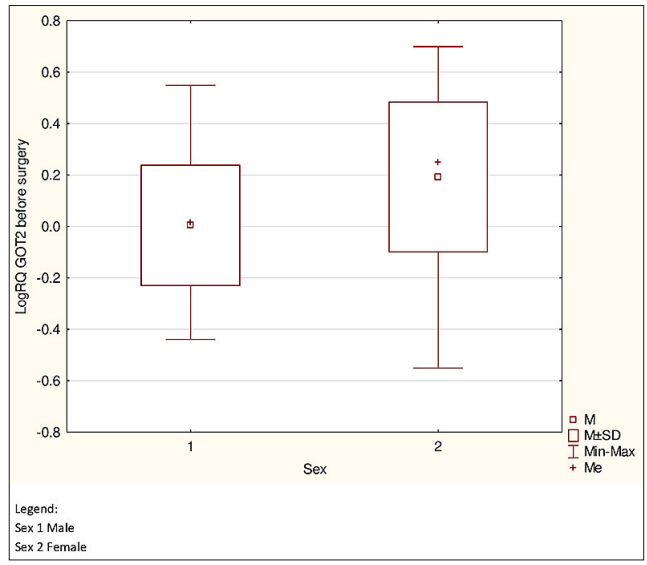

Figure 2. Differences in expression of mRNA LogRQ of the GOT2 gene between men and women in the study group before the procedure (p=0.0183).

DISCUSSION

This study presents the molecular effects of kynurenine function in patients operated on for non-small cell lung cancer.

Table 9. Statistical analysis of mRNA expression of the GOT2 gene in men and women in the study group. Application of the Mann-Whitney U test

Variable	Sum. rang Females	Sum. rang Males	U	Z	p	Z correct.	p	N Males	N Females
LogRQ *GOT2* before surgery	351.00	315.00	84.00	2.3584	0.0183	2.3584	0.0183	21	15
LogRQ *GOT2* on 3rd day after surgery	442.00	686.00	251.00	0.21884	0.8262	0.2188	0.8267	29	18
LogRQ *GOT2* on 6th day after surgery	413.00	668.00	203.00	0.8533	0.3934	0.8533	0.3934	32	16

The choice of this malignant tumour was not accidental, as it constitutes one of the most common causes of death, not only in Poland, but also worldwide. The high incidence of lung cancer, its secretive and insidious development in the human body, leads to late diagnosis which results in insufficient effectiveness of modern therapeutic methods.

The high mortality rate indicates that lung cancer is one of the deadliest killers in the modern population especially those individuals who struggle with nicotinism. The most common lung malignancy is large cell carcinoma, and the gold standard in cases of resectable non-small cell lung cancer is surgical resection of the tumour. Pulmonary parenchymal resection remains the main treatment for this type of early-stage lung malignancy. Stages I and II, and selected cases in stage IIIA, are treated by surgery. However, only a few patients qualify for such surgery, which constitutes about 15–20%. The proportion of five-year survival after surgical treatment ranges from 20–80%, depending on the stage, and is caused by sudden spread of the tumour. Diagnosis is based on the result of histopathological or cytological examination [25]. Radical surgical management due to the relatively aggressive course of the disease is limited to a small percentage of patients [26]. In the last decades, studies have emphasized that the results of surgical treatment have not improved significantly. Therefore, the current understanding of the clinical and molecular issues of lung cancer pathogenesis appears to be of utmost importance, as in the future it might assist in creating new perspectives for improving the results of its treatment. Molecular therapies based on, among others, Epidermal Growth Factor Receptor (EGFR) tyrosine kinase inhibitors, are currently used in approaching lung cancer. They are administered to those patients who have a mutation in the DNA sequence encoding the EGF receptor, which is located in exons 19 and 21 [27]. Drugs from the group that inhibit angiogenesis, e.g. monoclonal antibodies against vascular epithelial growth factor (VEGF) or VEGF receptor inhibitors, are used in advanced stages of large cell carcinoma. Studies of these drugs as components of combination treatment with surgical lung resection are still in progress. These studies emphasize the high relevance of kynurenine derivatives for targeted therapy. The kynurenine pathway has been gaining significant importance in medicine over the past decades. An important role in neural transmission has been discovered, that tryptophan is metabolized in the kynurenine metabolism pathway, which is implicated in the pathogenesis of depressive disorders. Some researchers (Mori Y et al.) report that kynurenine 3-monooxygenase is a key enzyme in the metabolism of KYN to 3-hydroxy kynurenine [25]. In laboratory mice, this can cause depression, and may increase levels of kynurenic acid, a KYN metabolite formed by kynurenine aminotransferases. The phenomenon occurs with the complicity of certain genes [28].

Another study examined the relationship between the levels of neuroprotective KYNA and neurotoxic kynurenine compounds among patients with the first episode of a restrictive form of Anorexia Nervosa. No significant changes in serum levels of these components were observed. However, an increase in CAT III expression was observed in the patients which, according to the authors, may have been caused by patients' excessive physical activity in the course of the study. However, the expression of genes in correlation with Fractalkine and the soluble cell adhesion molecule sICAM-1 which, according to the authors, modulates the Tryptophan/Kynurenine pathway, remains unknown and requires further research (Dudzinska E et al.). Fractalkine is a chemotactic factor that acts as an adhesion molecule, thus regulating leukocyte adhesion and migration. It also participates in cell apoptosis, free radical and cytokine release. Its full biochemical nature is currently at the stage of molecular research [26]. The goal set by researchers in the future is to use its therapeutic properties to combat numerous autoimmune diseases [19]. Other authors have presented the result of correlating the expression of genes encoding specific KYNA biosynthesis enzymes in lymphocytes with the occurrence of inflammatory bowel diseases (IBD). The results presented here showed that the presence of IBD is associated with increased expression of mRNA genes encoding KYNA biosynthesis enzymes in lymphocytes in patients participating in the study. Other extensive biochemical mechanisms, as well as environmental factors, may have influenced the final results. The authors suggest that KYNA in interaction with the aryl hydrocarbon receptor or the G-protein-coupled orphan receptor may participate in the counter-regulatory mechanism. It is the course of this process that may be important and affect the reduction of cytotoxicity, and thus the very process of IBD. (Dudzinska et al.). Learning about the response of KYNA biochemical pathways transformation will enable future understanding of the exact etiopathogenesis of IBD and thus improve the effectiveness of therapy [27].

Another interesting observation is that the kynurenine pathway and tryptophan catabolism are both linked by activation of the immune system to the action and signaling of neurotransmitters in the nervous system. Metabolites of the kynurenine pathway, kynurenic acid KYNA, were elevated in the brains of people with schizophrenia. In pathomorphological studies, the values of enzymes encoding mRNA genes, metabolites of the kynurenine pathway in the brain and plasma of the patients studied, differed according to the elevated levels of cytokines in schizophrenia patients as compared to the control group.

The current study provides key evidence for the important role of inflammation in a group of patients with schizophrenia. Further scientific studies on the molecular mechanism of the kynurenin pathway may prove a significant role of inflammatory processes in the etiology of schizophrenia in the future. The researchers conclusion that the occurring pro-inflammatory cytokines may induce a change in the biochemical properties of tryptophan to kinurenic acid in the brain periphery, may be worthy of attention [28].

The occurrence of their effect on the relationship between the increase in the concentration in the N-methyl-D-aspartate receptor antagonist KYNA, and the increase in mRNA genes encoding CAT enzymes, leads to greater synthesis activity of other enzymes in brain astrocytes, leading in turn to a loss of volume in the dorsolateral prefrontal cortex. According to Kindler J., et al.), this might impact the impairment of attention in chronic schizophrenia [28].

Kynurenine aminotransferases play an important role in ophthalmology. They are responsible for catalyzing kynurenic acid and their significant biological role in the healthy human cornea. The authors refer to gene expression data in the human eye structures that were extracted in laboratory procedures. All KAT isoforms were present on the corneal scrapings studied in patients with choroidal melanoma. All four enzymes were active in the corneal

scrapings studied. Matysik-Wozniak et al. found that kynurenine can be metabolized to KYNA in the corneal epithelium and its structural layers [29]. According to some authors, indoleamine 2,3-dioxygenase (IDO) plays a key role in immune response and escape in gastric cancer. Using RT- PCR and Western-blot analysis, the enzymatic activity of IDO was estimated by determining the concentrations of tryptophan and kynurenine in laboratory cell culture medium, using an amino acid analyzer. The effect of IDO on the cytotoxicity of T-lymphocyte-dependent proliferation was evaluated by Zhang et al. All the material was subjected to laboratory processing and observation. Conclusions from the study proved that IDO plays a key role in gastric cancer by inhibiting T-lymphocyte-mediated cytotoxicity and proliferation *in vitro* [30]. Sagan et al. proved some conclusions about the role of KYNA levels in patients with large cell carcinoma. The kynurenic acid KYNA is a byproduct of the kynurenine pathway, and in the future, its circadian measurement may provide the role of an indicator to demonstrate N0 or N1 trait in preparing a patient for surgery in all cases of large cell carcinoma.

Lymph node involvement before qualifying a patient for thoracic surgery is important for further treatment and prognosis. Lymph node metastasis in lung cancer is a significant, negative prognostic factor, usually impeding primary surgical resection. The authors, following their own study using high-performance liquid chromatography (HPLC), suggest that in the future circadian measurement of serum KYNA may constitute a diagnostic aid to facilitate the selection of candidates with large cell carcinoma for primary surgical resection of the lung parenchyma, or other treatments based on a robust assessment of metastases present in the lymph nodes. Studies, however, will require larger numbers of patients and careful analyses [31]. Currently, no detailed analysis exists in the literature of GOT2 gene mRNA expression of lung cancer.

CONCLUSIONS

Radical surgical resection of non-small cell lung cancer results in inhibition of GOT2 mRNA expression in lecukocytes. This information may be used as a target point for personalized therapies in lung cancer in the future. This may improve the quality of treatment and high survival rate, especially for patients from various environments, such as farmers, with an increased risk of lung cancer occurrence.

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