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Immunopathological and Stress-Related Markers in Patients with Non-Metastatic and Metastatic Pancreatic Cancer: An Exploratory Comparative Study

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Abstract: Pancreatic cancer is one of the most common malignant neoplasms, the incidence has increased in the population among patients who have recovered from various SARS-CoV-2 strains with subsequent post-COVID syndrome symptoms development. Given the heterogeneity of cancer development mechanisms within the population, the individual complex treatment implementation is essential for the correction of impaired immunometabolic processes. The aim is to assess pathological changes in humoral and cellular markers of innate and adaptive immunity in groups of patients with non-metastatic and metastatic pancreatic cancer. 64 patients (38 to 79 years) with pancreatic cancer were examined: group 1—non-metastatic (n = 44); group 2—metastatic (n = 20). The following research methods were used: ELISA (enzyme-linked immunosorbent assay), flow cytometry, immunoturbidimetry, spectrophotometry, light microscopy, impedance spectrometry. In first group patients had an increase in the C3 component complement, IL-1β, TNF-α, TLR4, TLR9 and DAMPs (Damage-Associated Molecular Patterns) cytotoxic oligonucleotide fraction. In second group patients had an increase in cortisol, C3 component complement, TLR9, oligopeptide and oligonucleotide DAMPs fractions and a maximum increase in the T regulatory cells CD3+CD4+CD25+CD127-. In both groups was increase in NK cells CD3-CD56+CD16+ and a wide spectrum of antinuclear antibodies to altered nuclear structures that acquired antigenic properties. In both groups, we identified cohorts of patients who with significant changes in the studied parameters set, had a decrease in serum electrical conductivity (on average by 45%). Individual changes in immunometabolic markers in patients of different ages with non-metastatic and metastatic pancreatic cancer have diagnostic and prognostic value.

Keywords: Pancreatic Cancer; Pathological Changes; Innate and Adaptive Immunity; Toll-like Receptors; Cytokines; NK Cells; Autoantibodies

1. Introduction

Pancreatic cancer is the 12th most commonly diagnosed malignancy and the sixth leading cause of cancer-related mortality worldwide, according to GLOBOCAN 2022 estimates [1]. Today, the incidence of this disease has

increased. This type of cancer has a low 5-year survival rate (approximately 10%) due to the diagnosis of the disease advanced stage [2]. Approximately 90% of pancreatic cancers have oncogenic mutations. In addition, new epidemiological features of the early cancer growth (in patients under 50 years) are observed against the background of the post-COVID syndrome (PCS) symptom complex and chronic stress, as well as due to the long-term post-stress reactions development, which are accompanied by an increase in oxidative reactions due to increased ROS and of mitochondrial dysfunction development [3].

It is known that the causes of cancer are a certain level of somatic mutations of oncogenes and suppressor genes. Epigenomic changes in some gene's expression are also important factors in the progression and the tumor process prevalence. Some mutated genes are important in transmitting signals for the passage of cells through immune checkpoints. There are mutations that mutate in many types of different localization cancer. In pancreatic tumors, repair genes are also disrupted, and this leads to the mutations high frequency accumulation in loci contained in DNA [4].

Previously, approaches to oncotherapy were characterized by a certain tumor-centricity, and only the effect of therapy directly on the tumor was considered appropriate without taking into account the effect of therapy on the patient's metabolic state [5]. Today, according to experts, cancer treatment goes beyond the tumor itself, since the influence of systemic factors is important—from disorders of the immune and nervous systems to the intestinal microbiome state and metabolic disorders in general [6]. Recently, the paradigm of the mutation's presence importance, which leads to chromosomal instability and contributes to the cell's malignancy, and epigenomic changes that contribute to the oncological process progression, is not considered as a single cause of the tumor's occurrence [7]. The attention of researchers, oncologists, immunologists is directed to the significance of changes in the tumor microenvironment factors, which include many different indicators, and affect the induction of an acute inflammatory reaction, the chronic inflammation development and cell aging. This supplemented paradigm of oncogenesis has contributed to the immunoresistance various disorders study in the conditions of the inflammatory reactions' development. It is important to consider the relationship between regulatory systems (immune, nervous, endocrine) that participate in tumor progression, and which will allow the results of basic research to be applied in clinical practice [8].

Currently, oncogenesis is defined not only as a local accumulation of mutations, but as a systemic dysfunction of the body, which makes possible further mutagenesis and mutated cells proliferation [9]. Immune dysfunction occupies one of the central places in cancer research, since evolutionarily the immune system must exercise control over aberrant cells. However, as it became known, the relationship between immune dysfunction and tumor is bidirectional, because the occurrence of tumors in the body also causes immune dysfunction, which, in turn, leads to further tumor growth [10].

Approaches to correct immune checkpoints are being developed in patients with cancer. Immunomodulatory approaches have been investigated as potential strategies for enhancing antitumor immune responses and improving survival outcomes in patients with pancreatic cancer [11]. There is data on pancreatic tumors that immune evasion can occur in the early stages of oncogenesis, in fact, even at the stage of malignant cells. There is evidence that in patients of different ages, the induction of the oncogenesis process differs both in its causes and in its course [3]. This may indicate that oncogenesis is dependent on the general functional state of the organism and the presence of the so-called SASP-phenotype syndrome (Senescence-Associated Secretory Phenotype). Goulart et al. believe that the key trigger of carcinogenesis in pancreatic cancer is the presence of long-term inflammatory signals in the initiation and progression of the tumor process and in the restructuring of the cellular microenvironment, which depends on changes in the function of T lymphocytes [12,13].

Epidemiological observations indicate the presence in some patients of a comorbid course of malignant pancreatic lesions, PCS symptoms manifestations and long-term persistence of pathogenic microbiota [14,15]. Taking into account the possible impact of all these factors on oncogenesis may be the basis for justifying immunotherapy. It is advisable to monitor the certain immune disorders presence to predict the tumor process possible progression and choose the tactics of complex personalized treatment of patients, taking into account the prevalence of impaired immunoresistance certain mechanisms.

Today there is no definite consensus on the factors that determine the causes and nature of the tumor process progression. It has not been possible to unify the patterns of oncogenesis and this disease course. And certainly, the study of microenvironmental factors, the role of the immune system in cancer progression and the role of the

microbiome in the chronic inflammatory response induction and cellular aging will be an addition to understanding cancer biology.

The aim of the study is to assess pathological changes in humoral and cellular markers of innate and adaptive immunity in groups of patients with non-metastatic and metastatic pancreatic cancer.

2. Materials and Methods

The study included 64 patients with non-metastatic and metastatic pancreatic cancer (pancreatic ductal adenocarcinoma, PDAC), aged 38 to 79 years (mean age 63.8 ± 0.9 years), who were treated at the clinic of the State Institution "V. T. Zaitsev Institute of General and Emergency Surgery of the National Academy of Medical Sciences of Ukraine" in the period 2025–2026 (the study is prospective). Among the examined patients, 48% were men (n = 31) and 52% were women (n = 33). Among the examined patients, two groups were distinguished depending on the non-metastatic and metastatic cancer: group 1—T3-4N0-2M0 (n = 44); group 2—T2-4N1-2M1 (n = 20). Patients gave informed consent before inclusion in the study. All participants were subjected to a clinical and anamnestic data collection, which included an assessment of history of pancreatitis or pancreatic necrosis, comorbidities, and the number of cases of laboratory-confirmed viral infection with SARS-CoV-2 different strains.

Staging of pancreatic cancer and corresponding clinical approaches to diagnosis, treatment, and follow-up were performed according to NCCN guidelines. Mandatory diagnostic methods were clinical examination, standard laboratory tests (with determination of the tumor marker CA19-9 both for diagnostic purposes and for the effectiveness of treatment (chemotherapy and surgery). According to the localization of PDAC, the distribution of patients was as follows: head of the pancreas (n = 36), body of the pancreas (n = 16), tail of the pancreas (n = 12).

Patients with metastasis (n = 20) and patients with locally unresectable (n = 8) forms of pancreatic cancer underwent palliative surgical interventions with subsequent chemotherapy after histological verification. Patients with resectable pancreatic cancer (n = 25) underwent radical surgical interventions. Patients with border resectable pancreatic cancer (n = 11) underwent chemotherapy with reassessment resectability and appropriate subsequent radical or palliative treatment.

The inclusion criteria for the study were: morphologically confirmed pancreatic cancer; absence of any interventions before admission for treatment; absence of acute infectious diseases at the time of examination; the ability to provide written informed consent for examination and treatment and fill out a questionnaire; a history of laboratory-confirmed COVID-19 disease (presence of antibodies and antigens) with subsequent assessment of symptoms of post-COVID syndrome.

The exclusion criteria were: the patient's refusal to participate in the study; previous chemotherapy or immunotherapy; severe mental or cognitive disorders that made it impossible to conduct the questionnaire; acute decompensated concomitant diseases or other conditions that could significantly affect the assessment of the studied indicators.

All study participants were surveyed to identify symptoms of post-COVID syndrome: A—disorders of the musculoskeletal system; B—disorders of the respiratory system; C—neuro-cognitive disorders; D—purulent-inflammatory processes; E—cardiovascular disorders; F—disorders of the gastrointestinal tract.

This study was carried out in accordance with the principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects" (as revised in 2013). The study protocol was approved by the local bioethics committee of the State Institution "V.T. Zaitsev Institute of General and Emergency Surgery of the National Academy of Sciences of Ukraine".

The material for the study was serum and formed blood elements of patients with pancreatic cancer. The detected changes in immune markers were compared with reference values, which correspond to their average values in healthy individuals. For reference values, we took the average values of the studied parameters obtained in our laboratory for a long time (five years) in healthy individuals (up to 400).

The content of C3 complement component was determined by immunoturbidimetry (Stat-Fax-1900, USA) based on the precipitation reaction. The optical density, which was proportional to the C3 component concentration in the sample, was measured at a wavelength of 340 nm. A Dialab reagent kit (Austria) was used.

Determination of cortisol, toll-like receptor 4 (TLR4) and 9 (TLR9), cytokines (IL-1β, TNF-α, IL-2) in blood serum was performed by solid-phase enzyme-linked immunosorbent assay test systems using monoclonal antibodies adsorbed on polystyrene plates (Fine-test, China). The formed "antigen-antibody" complex was detected

using a conjugate, the peroxidase of which catalyzes the cleavage of the substrate (hydrogen peroxide), causing a change in the color of the indicator. The concentration of the substance to be determined was judged by the change in color. Optical density measurements were performed at a wavelength of 450 nm (StatFax 3200, USA).

The cellular component of innate immunity was assessed by studying the phagocytic activity of neutrophils in oxygen-independent phagocytosis by quantitatively determining the absorption and digestion capacity of neutrophils in relation to the antigen (Saccharomyces cerevisiae). The phagocytic index (PhI, %) was determined as the percentage of neutrophils that absorb antigen and participate in phagocytosis, from their total number; phagocytic number (PhN, conventional units)—the average number of microorganisms absorbed by one neutrophil (the fraction of dividing the total number of consumed antigens by the number of cells that entered phagocytosis). The main indicator for assessing the intensity of oxygen-independent phagocytosis was the phagocytosis completion index (PhCI), which characterizes the digestion capacity of phagocytes for antigen processing. Light microscopy (Olympus BX-53, Japan) at a magnification of ×1,000 was used to analyze the stained preparations [16].

Formed blood elements—the number of monocytes, lymphocytes, neutrophils were determined by cytometric method. Blood was collected from monovets (K3 EDTA) for further analysis of the different cells number on an automatic analyzer Mindray BC-2800 Vet (USA).

Flow cytometry. The analysis was performed 2 h after blood sampling using test tube with the K3 EDTA according to the standard protocol. In each sample at least 5000 cells were analyzed. Various subpopulations were determined: CD3-CD56+CD16+ (NK cells), CD3+CD4+CD25+CD127- (T regulatory cells) using appropriate monoclonal antibodies and dyes: CD3-ECD, CD4-FITC, CD16-FITC, CD56-PC7, CD25-PC5, CD127-PE (Beckman Coulter, USA). Cells are stained in polystyrene round-bottom 12 × 75 mm Falcon tubes. We added 100 μL of cell suspension and 10 μL of the certain primary labeled antibody to each tube and incubate 15 min at room temperature in the dark. To remove erythrocytes of sample preparation no-wash technology using OptiLyse C (Beckman Coulter, USA)—500 μL per tube. Then incubate for at least 15 min at room temperature; centrifuge at 1,500 rpm for 5 min at 4 °C and discard supernatant. After this, 500 μL of FACS buffer was added. The analysis was carried out immediately after the preparatory stages. For correct exclusion cells that did not meet the parameters from the analysis zone, the necessary logical constraints were introduced into the particle distribution histogram for low-angle, side scintillation (SSC). The evaluation of expression level of surface receptors was performed at a mean intensity of fluorescence. Samples were analyzed on the flow cytometer Cytomics FC500 (Beckman Coulter, USA). Data were processed by Software Version: CXP 2.2.

Molecules formed during damage were assessed in blood serum by the content of DAMPs (Damage-Associated Molecular Patterns) fractions spectrophotometrically (Shimadzu UV-2600, Japan) at different wavelengths: oligopeptide fraction—238 nm and oligonucleotide fraction—260 nm [17].

The autoimmune component was assessed by the presence of antinuclear antibodies (ANA) of different specificities. Serum specific antinuclear antibodies (ANA) were detected by indirect immunofluorescent analysis (Olympus BX53, Japan) using monoclonal antibodies (MAbs) labeled with fluorescein (FITC) and glasses with biochip reaction zones, the surface of which is coated with a substrate: HEp-2 and primate liver sections (EUROIMMUN Reagent Kit, Germany). Serum of patients was applied to glasses with biochips and incubated. Specific antibodies of the IgA, IgG and IgM classes present in the positive samples bound to the chip antigens. Then, bound antibodies were detected by fluorescent staining after incubation of slides with FITC-labeled antibodies to the corresponding human immunoglobulins, antinuclear antibodies. Luminescence specificity was evaluated at ×400 magnification.

Bioindication of the cytotoxic factors totality in blood serum was performed using a culture of the unicellular alga Dunaliella viridis by light microscopy at a magnification of ×400 (Olympus BX-53, Japan). After co-incubation of the cell suspension of the bioindicator Dunaliella viridis with the studied serum, cells with altered morphological (cell shape) and functional (loss of mobility) characteristics were counted. The cell aggregates formation and the exometabolite release were also observed at high levels of cytotoxic serum factors. The serum cytotoxicity coefficient (Cc) was calculated to analyze the degree of serum integral cytotoxicity [18].

For the current screening of the impedance characteristic of the serum cytotoxic factors set, the indicator of blood serum conductivity was used, which was measured using a vector analyzer ZNB 40 “Rohde & Schwarz” (Austria). The conductivity of the measuring cell contents is the reciprocal of the active resistance (1/R), and is determined by the properties of certain serum substances. The results were presented in the form of specific conductivity values (σ) [19].

To determine the relationship between immunological disorders and changes in screening tests (integral cytotoxicity in the *Dunaliella viridis* test and serum conductivity), patients in two groups were divided into four selective cohorts depending on the level of conductivity: cohort "1-1" (n = 15); cohort "1-2" (n = 29); cohort "2-1" (n = 8); cohort 2-2 (n = 12). The limiting value of electrical conductivity is 0.85 S/m.

Statistical analysis. Data analysis was performed using Statistica 6.1 program (StatSoft Inc., USA) and Microsoft Excel 2019. Mean values (x) and standard deviations (SD), statistical significance levels were calculated. Data normality was assessed using the Shapiro-Wilk test. One-way ANOVA was used to assess differences between several independent groups. Benjamini–Hochberg false discovery rate correction (FDR) was used to control the risk of multiple testing and reduce the likelihood of false positive results when performing pairwise comparisons. Adjusted *p*-values were used to interpret the statistical significance of the results. The difference between groups was considered statistically significant at $p < 0.05$.

3. Results

According to the questionnaire, all patients had different frequencies of post-COVID syndrome (PCS) symptoms. Analysis of the various PCS symptoms occurrence showed that all examined patients (100%) had symptoms associated with disorders of the gastrointestinal tract. Some patients had a history of pancreatitis. Symptoms of neuro-cognitive disorders and disorders of the musculoskeletal system were also detected with a high frequency. Also, patients of both groups with PCS symptoms had disorders associated with the respiratory system with the same frequency (14%). Cardiovascular system disorders were detected in only 43% of the elderly patients in second group (**Table 1**).

Table 1. Frequency of post-COVID syndrome (PCS) symptoms in patients with non-metastatic and metastatic pancreatic cancer.

Symptom Group	Frequency of Certain PCS Symptoms Presence (in %) in Patients with Pancreatic Cancer	
	Group 1 Non-Metastatic (n = 44)	Group 2 Metastatic (n = 20)
A. Musculoskeletal disorders	57	64
B. Respiratory disorders	14	14
C. Neurocognitive disorders	79	85
D. Purulent-inflammatory processes	-	-
E. Cardiovascular disorders	-	43
F. Gastrointestinal disorders	100	100

All examined patients had pathological changes in humoral factors. These changes in humoral indicators occurred against the background of a significant increase in the stress hormone cortisol: in group 1, an increase of 32% was found, in group 2, a more significant increase of this hormone by 55%, respectively, relative to the reference level (560.0 ± 32.1 pg/mL) (**Figure 1**).

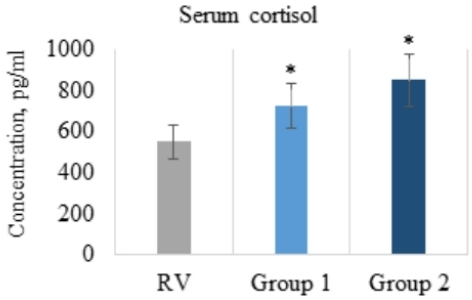


Figure 1. Serum cortisol concentration in groups of patients with non-metastatic (Group 1, n = 44) and metastatic (Group 2, n = 20) pancreatic cancer; * $p < 0.05$ compared with reference values (RV).

The serum concentration of the C3 component complement was increased in two groups relative to reference values (1.05 ± 0.41 g/L): in the first group, the C3 component was increased to 2.9 ± 0.4 g/L, and in second group, the increase in this protein was lower (1.7 ± 0.3 g/L) (**Figure 2**).

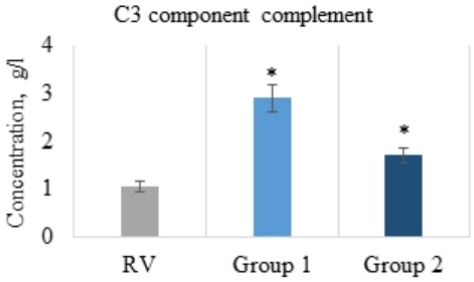


Figure 2. Concentration of C3 component complement in groups of patients with non-metastatic (Group 1, n = 44) and metastatic (Group 2, n = 20) pancreatic cancer; * $p < 0.05$ compared with reference values (RV).

The content of pro-inflammatory IL-1β in patients without metastasis was increased to 11.1 ± 4.3 pg/mL, and in patients with metastasis this indicator was 6.9 ± 0.3 pg/mL with reference values of 5.4 ± 1.3 pg/mL (**Figure 3a**).

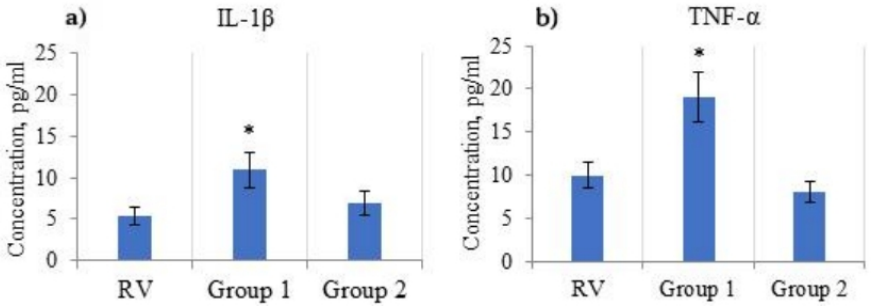


Figure 3. The content of IL-1β (**a**) and TNF-α (**b**) in patients with non-metastatic (Group 1, n = 44) and metastatic (Group 2, n = 20) pancreatic cancer; * $p < 0.05$ compared to reference values (RV).

A significant change in serum TNF-α concentration was found in the first group (patients without metastasis), where this indicator was 19.1 ± 0.9 pg/mL with reference values of 10.1 ± 0.4 pg/mL. In the second group (patients with metastatic pancreatic cancer), serum TNF-α content was below reference values and was 8.0 ± 3.7 pg/mL (**Figure 3b**).

Analysis of changes in the pro-inflammatory serum factors content in the microenvironment of pancreatic tumors in non-metastatic and metastatic cancer depending on age showed different trends. In young patients in the first group (patients without metastasis), a significant increase in IL-1β and a low level of TNF-α were observed (**Figure 4a**). In the second group (patients with metastatic pancreatic cancer) 2, on the contrary, in older patients with metastasis, a tendency to a smooth increase in the level of TNF-α was observed. But patients in this group over 70 years of age had a trend towards a decrease in IL-1β and TNF-α (**Figure 4b**).

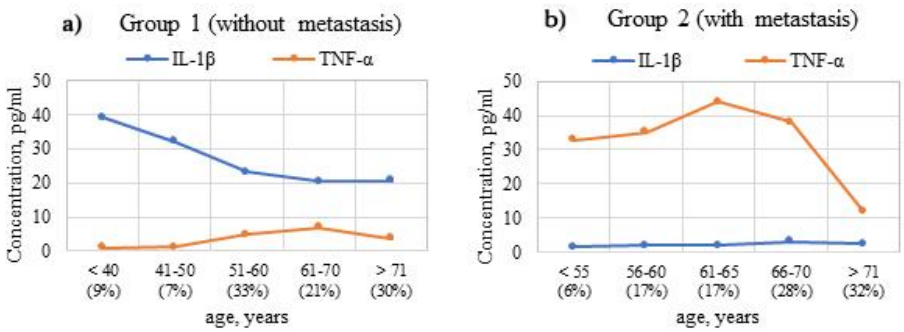


Figure 4. Relationship changes between IL-1β and TNF-α depending on the age in patients with non-metastatic (**a**) Group 1, n = 44 and metastatic (**b**) Group 2, n = 20 pancreatic cancer.

Patients without metastasis of first group had an increase in IL-2 at the level of (128.8 ± 34.3) pg/mL, which was 8 times higher than the reference value (16.1 ± 3.4 pg/mL). Patients with metastatic cancer of second group had the IL-2 content (18.1 ± 6.8 pg/mL) at the reference values. It should be noted that examined patients had the age-dependent content of IL-2. A pronounced increase in IL-2 was detected in middle-aged and elderly patients in group 1 (**Figure 5**).

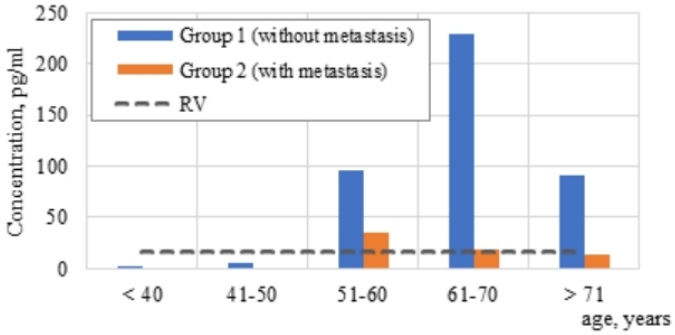


Figure 5. Changes in IL-2 levels in patients with non-metastatic (Group 1, n = 44) and metastatic (Group 2, n = 20) pancreatic cancer. RV: reference values.

The expression of TLR receptors to various antigens differed in two groups. The level of TLR4 expression in the first group (without metastasis) was significantly increased (11.9 times) relative to the reference values (2.0 ± 0.9 ng/mL). In the second group (with metastasis), the response to infectious antigens was absent and this indicator was below the reference values (**Figure 6a**).

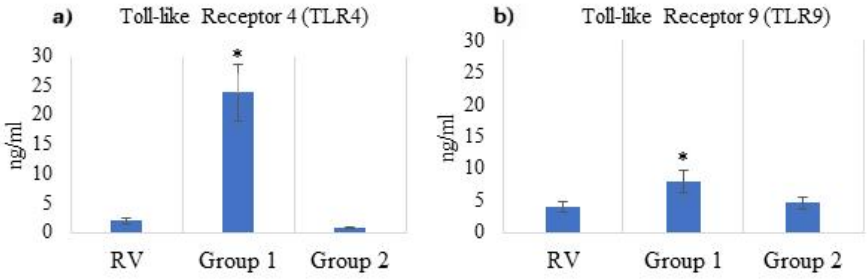


Figure 6. Levels of TLR4 and TLR9 in patients with non-metastatic (Group 1, n = 44) and metastatic (Group 2, n = 20) pancreatic cancer; * $p < 0.05$ compared to reference values (RV).

An increase in the intracellular receptor TLR9 was found in both groups, but in the first group this indicator was higher than in the second group and was 8.06 ± 7.4 ng/mL relative to the reference values (4.0 ± 0.5 ng/mL), and in the second group, 4.7 ± 0.6 ng/mL (**Figure 6b**).

In both groups an increase in the DAMPs content was found. In the first group (without metastasis), the DAMP oligopeptide fraction content was 1.22 ± 0.16 units E, at reference values of 0.62 ± 0.09 units E, and the DAMP oligonucleotide fraction content was 0.64 ± 0.12 units E, at a reference level of 0.24 ± 0.05 units of E. In the second group (with metastasis), an increase in the DAMPs cytotoxic fractions was also found—oligopeptide fraction up to 1.074 ± 0.073 units E, and oligonucleotide fraction up to 0.514 ± 0.170 units E (**Figure 7a,b**).

In the examined groups of patients (M0 and M1), the general analysis of the blood cellular composition showed that the level of monocytes on average did not exceed the reference values in both groups. In the first group, a decrease in the level of lymphocytes was found, while in the second group this indicator had a high degree of variability: in 51% of patients this indicator was reduced (up to 12.7 ± 1.9%), in 44% of patients the number of lymphocytes did not differ from the reference values (22.3 ± 2.4%), and only in 5% of patients it exceeded the reference values (39.5 ± 2.8%). In both groups, a significant increase in neutrophils was found, while in the second group a more significant increase in this indicator was found (up to 30.0 ± 1.9%) (**Figure 8**), which indicates the activation of the

synthesis of granzyme enzymes by phagocytic cells. In the second group (with metastasis), a tendency to change this indicator was also found.

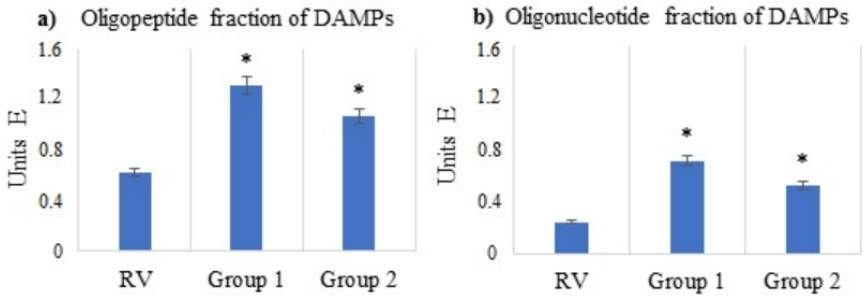


Figure 7. The content of DAMPs fractions (oligopeptide and nucleotide) in groups of patients with non-metastatic (Group 1, n = 44) and metastatic (Group 2, n = 20) pancreatic cancer; * $p < 0.05$ compared with reference values (RV).

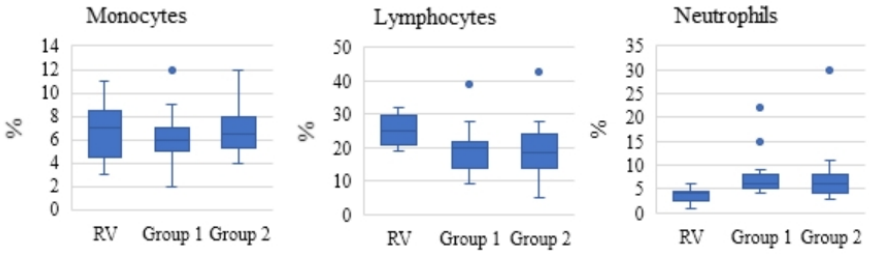


Figure 8. Levels of monocytes, lymphocytes and neutrophils in groups of patients with non-metastatic (Group 1, n = 44) and metastatic (Group 2, n = 20) pancreatic cancer; * $p < 0.05$ compared with reference values (RV).

The content of innate immune killer cells (NK cells) had high variability in both groups, but on average these cells in the second group were more activated (up to 30.1 ± 4.3%) with reference values of 17.5 ± 2.5% (**Figure 9**).

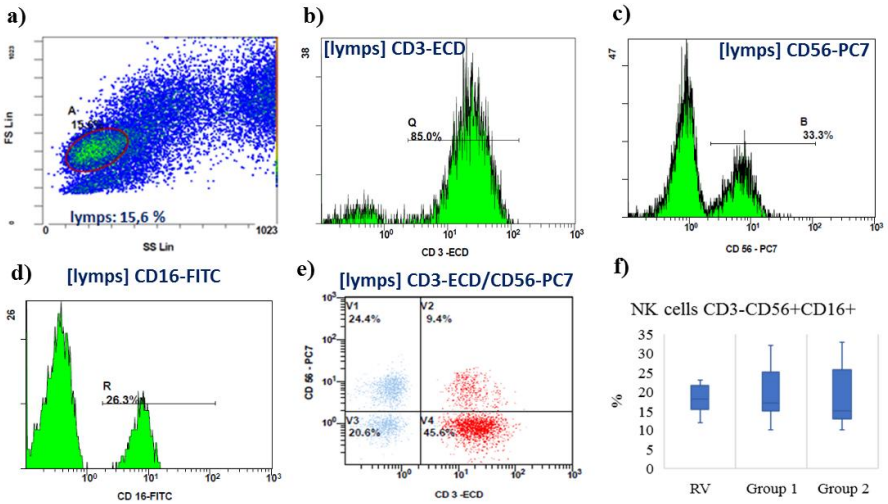
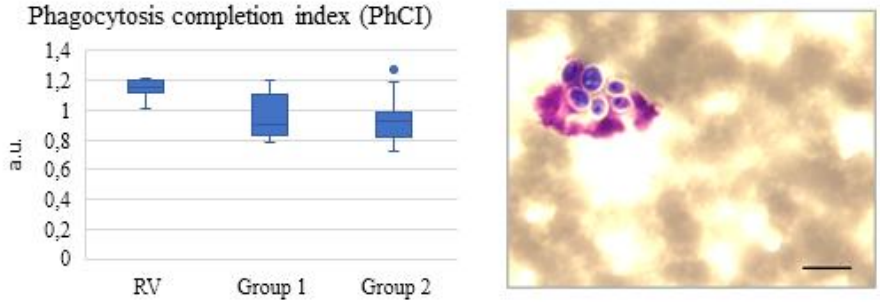


Figure 9. Gating strategy for determination of NK cells CD3-CD56+CD16+. (**a**) gating FSC versus SSC, gating of lymphocytes and identification of (**b**) CD3+; (**c**) gating of population and identification of CD56+; (**d**) gating of population and identification of CD16+; (**e**) population of NK cells CD3-CD56+CD16+; (**f**) NK cell content in groups of patients with non-metastatic (Group 1, n = 44) and metastatic (Group 2, n = 20) pancreatic cancer. The number of cells with the CD3-CD56+CD16+ phenotype is indicated by the V1 quadrant (**e**). The example shown for patient with metastatic pancreatic cancer (group 2). RV: reference values.

Analysis of the phagocytic cells functional state of innate immunity showed a low level of antigen processing in both groups, which indicates a low efficiency of the digestive properties of phagocytic neutrophils granzyme enzymes (**Figure 10a,b**).



(**a**) (**b**)

Figure 10. Oxygen-independent neutrophil phagocytosis: (**a**) antigen processing completion degree in patients with non-metastatic (Group 1, n = 44) and metastatic (Group 2, n = 20) pancreatic cancer. * $p < 0.05$ compared with reference values (RV). a.u.: arbitrary units. (**b**) phagocytic neutrophil at the stage of intracellular processing of antigens. Incomplete digestion of antigens. Scale bar 10 μm.

The expression of T regulatory (Treg) cells with the phenotype CD3+CD4+CD25+CD127- significantly differed between groups and compared with reference values (5.2 ± 0.6%). In the first group (without metastasis), Treg expression was reduced by 23% compared with reference values, and in the second group of patients with metastasis, on the contrary, a significant increase in Treg cell expression by 153% was found.

The frequency of antinuclear autoantibodies (ANA) occurrence in patients without metastasis (group 1) was 34.2%, and in patients with metastasis (group 2), 61.0%. A different repertoire of ANA was found in both groups of patients with pancreatic cancer. The frequency of ANA occurrence to native DNA in both groups was approximately the same, and was 13.3% and 15.0%. These antibodies negatively affect the regulation of proliferation and apoptosis of cells. Also, in both groups, autoantibodies to the transcription factors TIF-1β and TIF-1γ were found, which occurred with a frequency of M0 8% and M1 10% (**Figure 11a,b**).

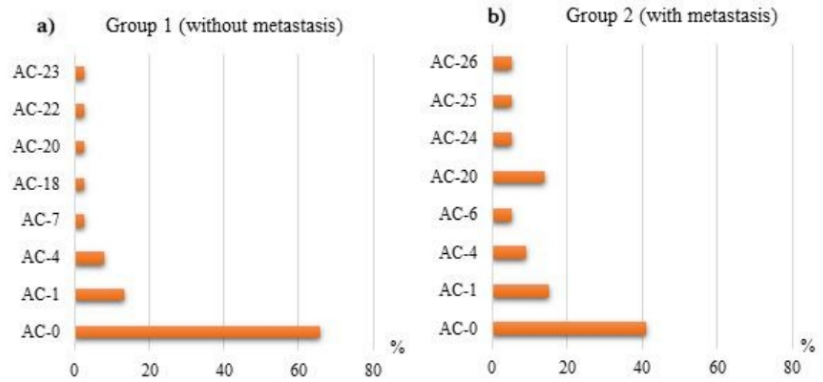
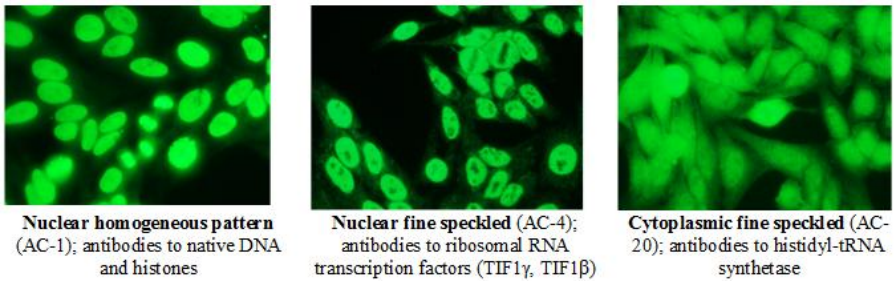


Figure 11. Frequency of antinuclear autoantibodies ANA in patients with non-metastatic pancreatic cancer (**a**) Group 1 and in patients with metastatic pancreatic cancer (**b**) Group 2. Nuclear, cytoplasmic patterns and association with specific antigens according to Chan et al. [20]. Codes AC (AC: anti-cell pattern) in accordance with the International Consensus on ANA (Antinuclear antibodies) Patterns (ICAP) are presented.

Note: AC-1:antibodies to native DNA and histones; AC-4: antibodies to ribosomal RNA transcription factors (TIF1γ, TIF1β); AC-6: antibodies to Sp-100 proteins and PML proteins; AC-7: antibodies to Cajal body antigens; AC-18: antibodies to processing P-bodies and glycine/tryptophan-like bodies; AC-20: antibodies to histidyl-tRNA synthetase; AC-22: antibodies to Golgi apparatus protein structures; AC-23: antibodies to inosine monophosphate dehydrogenase-2 (IMPDH2); AC-24: antibodies to centriole proteins (pericentrin, ninein, Cep250, Cep110); AC-25: antibodies to kinesin-like protein HsEg5 of the division spindle; AC-26: antibodies to the protein structure of the cell division spindle NuMA.

All patients had a high frequency of ANA cross-reactivity to native DNA (AC-1), to the transcription factors

TIF1β and TIF1γ (AC-4), and to Jo-1/histidyl-tRNA synthetase (AC-20). Specific types of fluorescence are presented in the **Figure 12**.



Nuclear homogeneous pattern (AC-1); antibodies to native DNA and histones

Nuclear fine speckled (AC-4); antibodies to ribosomal RNA transcription factors (TIF1γ, TIF1β)

Cytoplasmic fine speckled (AC-20); antibodies to histidyl-tRNA synthetase

Figure 12. Specific types of ANA fluorescence with high frequency in patients with pancreatic cancer.

In the presence of ANA to native DNA, a homogeneous nuclear type of fluorescence (AC-1) is observed. The presence of ANA to the transcription factors TIF1β and TIF1γ is characterized by a nuclear fine-grained type of fluorescence (AC-4). The presence of ANA to Jo-1/histidyl-tRNA synthetase is indicated by a cytoplasmic fine-grained type of luminescence in blood serum (AC-20) (**Figure 12**).

For an integrated assessment of immunometabolic changes, two screening tests were used: the reaction of the *Dunaliella viridis* bioindicator to blood serum components and the degree of changes in serum electrical conductivity.

All examined patients had an increase in the integral serum cytotoxicity index (in test with *Dunaliella viridis*) compared to reference values (1.5 ± 0.2 a.u.): in the first group to 4.3 ± 1.2 a.u., in the second group to 4.1 ± 2.7 a.u.

A change in the electrical conductivity of blood serum was detected, which is a physical indicator of the serum factors cytotoxicity degree and characterizes its impedance [21]. In patients of the first group, the electrical conductivity of serum factors was below the reference values (1.1 S/m) and ranged from 0.40 to 0.91 S/m (on average, it was 0.81 S/m), and a significant decrease in electrical conductivity in the range from 0.4 to 0.6 S/m was detected in 26% of the examined patients without metastasis. In the second group, the electrical conductivity of serum factors was in the range from 0.63 to 0.93 S/m (on average, 0.83 S/m and did not differ from the indicator of the first group), and the number of cases of a significant decrease in electrical conductivity was detected in 33% (electrical conductivity in the range from 0.63 to 0.75 S/m).

To characterize the degree of pathological changes in microenvironmental factors in patients, the value of blood serum impedance was studied, which was judged by changes in serum conductivity. In order to identify the relationship between the magnitude of immunopathological disorders and the value of serum conductivity, patients were divided into selective cohorts depending on the level of conductivity (**Table 2**).

Table 2. Indicators in patients with non-metastatic (Group 1, n = 44) and metastatic (Group 2, n = 20) pancreatic cancer in selective cohorts depending on the level of serum conductivity.

Parameter	Reference Values	Group 1, n = 44 Non-Metastatic Process		Group 2, n = 20 Metastatic Process		*p*-Value	q-Value (BH)	Statistical Significance (FDR < 0.05)
		Selective Cohorts						
		"1-1" n = 15	"1-2" n = 29	"2-1" n = 8	"2-2" n = 12			
	σ – N	σ – ↑ (>0.85)	σ – ↓ (< 0.85)	σ – ↑ (>0.85)	σ – ↓ (< 0.85)			
Electrical conductivity (σ), S/m	1.1 ± 0.1	0.97 ± 0.07	0.74 ± 0.05	0.99 ± 0.08	0.75 ± 0.08	0.016	0.037	NS
IL-1β, pg/mL	11.0 ± 2.3	7.4 ± 1.7	18.8 ± 0.01	11.3 ± 2.9	0.4 ± 0.1	0.00024	0.00080	Sig.
IL-2, pg/mL	15.0 ± 1.1	327.8 ± 52.	35.5 ± 4.5	39.5 ±15.3	20.0 ± 2.7	0.00051	0.00128	Sig.
TNF-α, pg/mL	10.0 ± 2.4	2.9 ± 0.9	6.5 ± 1.1	7.5 ± 0.7	7.3 ± 1.1	0.147	0.245	NS
TLR4, ng/mL	2.0 ± 0.5	42.3 ± 16.5	0.95 ± 0.06	0.6 ± 0.01	0.3 ± 0.1	0.000044	0.00044	Sig.
TLR9, ng/mL	4.0 ± 0.5	11.0 ± 1.7	4.8 ± 1.7	2.6 ± 0.8	6.3 ± 2.4	0.00048	0.00128	Sig.
Cortisol, pg/mL	405.0 ± 21.1	742.0 ± 57.4	880.9 ± 94.6	1052.1 ± 196.3	810.3 ± 79.8	0.486	0.607	NS
C3 component, g/L	1.05 ± 0.20	1.53 ± 0.81	3.37 ± 0.92	1.26 ± 0.50	2.33 ± 0.41	0.015	0.030	Sig.
DAMP oligonucleotide fraction, units E	0.24 ± 0.02	0.55 ± 0.15	0.69 ± 0.32	0.46 ± 0.11	0.57 ± 0.22	0.863	0.954	NS
Cc (*Dunaliella viridis*), a.u.	1.5 ± 0.6	3.7 ± 1.9	4.7 ± 1.1	3.6 ± 0.7	3.6 ± 1.6	0.451	0.607	NS

Note: *p*-values were obtained using one-way ANOVA; q-values were adjusted using the Benjamini–Hochberg procedure to control the false discovery rate (FDR) at 0.05. Selective cohorts with low and very low levels of electrical conductivity were identified in groups of patients with non-metastatic (Group 1) and metastatic (Group 2) pancreatic cancer. a.u.—arbitrary units. Sig.—significant; NS—not significant.

In cohort "1-1" of the first group, the electrical conductivity was at moderately reduced level. Studies of im-

munopathological factors of the microenvironment showed that the IL-1β content in cohort "1-1" was within the range of reference values. In cohort "2-1" (from the second group), IL-1β was 11.3 ± 2.9 pg/mL. The concentration of IL-2 in cohort "1-1" was increased 22 times and 2.6 times in cohort "2-1", respectively. The content of TNF-α in both cohorts ("1-1" and "2-1") was below the reference values. Patients of cohort "1-1" had an increase in TLR4 levels (21 times) relative to the reference values (2.0 ± 0.9 ng/mL) and an increase in TLR9 levels (2.8 times) relative to the reference values (4.0 ± 0.5 ng/mL). In cohort "2-1", the expression of TLR4 and TLR9 was below the reference values. In both cohorts, the oligonucleotide fraction of DAMP exceeded the reference values, more significantly in cohort "1-1" by 2.3 times (0.55 ± 0.15) units E, at the reference level (0.24 ± 0.05) units E, in cohort "2-1" this indicator was increased by 1.9 times. The integral cytotoxicity coefficient, which was determined using the *Dunaliella viridis* bioindicator, was increased in both cohorts by 2.5 times compared to the reference values (1.5 ± 0.6 a.u.) (**Table 2**).

When analyzing immunopathological factors in selective cohorts with a very reduced level of electrical conductivity, some differences were found in comparison with selective cohorts of the moderately reduced level group. The content of IL-1β in cohort "1-2" was higher than the reference values and was 18.8 ± 0.01 pg/mL, and in cohort "2-2" it was 0.4 ± 0.1 pg/mL. The level of IL-2 in cohorts with a reduced level of electrical conductivity was higher than the reference values and was in cohort "1-2" 35.5 ± 4.5 pg/mL, and in cohort "2-2" 20.0 ± 2.7 pg/mL. The content of TNF-α was within the reference values. Also, the concentration of TLR4 did not exceed the reference values, and the concentration of TLR9 was increased in cohort "2-2" by 37%. The concentration of cortisol was equally twofold increased in the cohorts with a reduced level of electrical conductivity regardless of the metastasis's presence. The concentration of the C3 component complement was significantly increased against the background of a decrease in electrical conductivity and was in cohort "1-2" 3.37 ± 0.9 g/L, and in cohort "2-2" 2.33 ± 0.4 g/L. The cytotoxic oligonucleotide fraction DAMPs was the highest in the cohort "1-2" 0.69 ± 0.32 units E, in cohort "2-2" 0.57 ± 0.22 units E. The integral cytotoxicity coefficient was maximum in cohort "1-2" and was 4.7 ± 1.1 a.u., and in cohort "2-2" it was also above the reference values and was 3.6 ± 1.6 a.u. (**Table 2**).

4. Discussion

Pancreatic cancer is asymptomatic at the beginning of the disease, and then has a high mortality rate. It is known that one of the main causes of carcinogenesis may be the occurrence of damage in the synthesized DNA chain, which can lead to genetic instability of the DNA nucleotide repair system genes. The *KRAS* oncogene is known to have the highest mutation rate among all types of malignant tumors. This gene encodes the protein of the same name, which is part of the RAS/MAPK (mitogen-activated protein kinase) signaling cascade, which is an important chain of signal transmission from the cell membrane to the nucleus [22]. Silencing or genetic ablation of oncogenic KRAS in pancreatic cancer cells can result in cell-cycle arrest, cell death, and inhibition of proliferation *in vitro* [23]. *KRAS* activation occurs when epidermal growth factor (EGF) binds to tyrosine kinase receptors [24].

In addition to oncogenes, mutations in suppressor genes are important. The cause of pancreatic cells malignancy, along with somatic mutations, are mutations in several suppressor genes, such as *SMAD4*, *TP53*, and *CDKN2A*/*INK4A* (Cyclin dependent kinase inhibitor 2A/Inhibitors of CDK4), which are important in the pancreatic cancer progression. Deletions of the *SMAD4* suppressor gene contribute to pro-oncogenic responses by reducing inhibition of growth factor beta TGF-β [25]. Loss of CDKN2A function contributes to pancreatic cancer progression and metastasis by disrupting cell-cycle checkpoint control and promoting an aggressive tumor phenotype [26]. Disruption of the expression of the *BRCA1* (BReast CAncer gene 1) and *BRCA2* (BReast CAncer gene 1) genes, which belong to the group of tumor suppressor genes, initiate different repair mechanisms, but are capable of mutations. As a result, the function of repairing damaged DNA is disrupted and genome instability increases. Georgy et al. believe that the deficiency of the DNA repair mechanism leads to microsatellite instability and a significant risk of cancer development [27]. The microsatellite instability prevalence due to DNA repair defects leads to a high frequency of mutations in loci containing microsatellites. Microsatellite instability in cells is formed during DNA replication and, when the repair system is deficient, such changes in microsatellites accumulate. Cells microsatellite instability manifests itself in the form of the neoantigens large number accumulation as a result of transcription from these DNA regions [28]. But as is known, the tumor-centric paradigm is today complemented by the importance of changes in microenvironmental factors in the progression of the oncological process. Oncogenesis is promoted not only by the activation of oncogenes and genetic mutations, but also by the chronic inflammatory reactions' presence, which

can be induced by bacterial and viral infections due to their long-term persistence. Wang et al. showed that acute and chronic inflammatory reactions alter the tumor microenvironment, which includes the interaction of cells with a network of cytokines and chemokines, as well as the interaction between tumor cells, stromal cells, immunocompetent cells, including endothelial inflammation [29].

In this study, all patients with pancreatic cancer were found to have bacterial infection and post-COVID syndrome (PCS) symptoms. Against the background of PCS symptoms, pathological changes were observed with varying frequency, gastrointestinal tract, musculoskeletal system damage, cognitive impairment and long-term pathology, which was accompanied by certain changes in immunoresistance [30]. Patients with pancreatic cancer also have altered weakened immunity against the background of long-term stress, which is confirmed by rather high levels of cortisol in the examined patients of both groups.

Inflammation and onco-regeneration are interrelated processes in which the immune system plays a decisive role. Cell cycle disruption, genomic instability, and failure to eliminate tumor cells occur under conditions of constant exposure to inflammatory signals. This exposure will largely determine the subsequent ontogenesis of the cell: its elimination, transition to a state of aging, or degeneration with the accumulation of mutations. According to Phan & Croucher, the immune system loses its ability to provide rapid, effective antitumor responses and shifts toward prolonged, low-intensity inflammation [31].

The close anatomical connection between the pancreas and the gastrointestinal tract via the pancreatic duct is the basis for a bidirectional relationship between the gut microbiota and the pancreas (the "pancreas-microbiota axis") [32]. Local and systemic microbial populations can significantly influence the immune response and metabolic potential of the microenvironment. Changes in the gut microbiota (higher levels of *Flavobacteria*, *Proteobacteria*, *Actinobacteria*, *Alphaproteobacteria*, *Sphingobacteria*, *Staphylococcus aureus*, *Candida*, *Klebsiella*, *Helicobacter pylori* in the stomach) can provide inflammatory stimuli that contribute to the chronic pancreatitis progression, and of course the risk of developing pancreatic cancer [33,34]. Some patients had a history of comorbid gastrointestinal diseases against the background of chronic infection with *Helicobacter pylori* of the oncogenic strain *CagA* [3]. Changes in the body's microbiota (PAMPs), as well as microbial toxins, increased ROS and mitochondrial DNA damage lead to the NLRP3 inflammasome activation, less often NLRC4, which is a multiprotein complex of innate immunity, with subsequent activation of caspase-1 [35].

All examined patients of the two groups had a significant increase in the C3 complement component, which contributes to the enhancement of mediated, damage to cell membranes and tissues, including endothelial cells, and increased their lysis. Ajona et al. believe that complement activation can also lead to intravascular hemolysis [36]. An increase in the level of the C3 component can have a dual effect, namely an onco-suppressive effect at the beginning of the oncological process, which manifests itself, due to the opsonizing function, in the phagocytosis stimulation, or a pro-oncogenic effect in the event of phagocytosis deficiency (this was observed in both groups, especially in the second group of patients with metastasis).

The cellular factor of innate immunity, natural killer NK cells play a role in controlling tumor growth and immune surveillance of the tumor [37]. In both groups, we observed an increase in the NK cells CD3-CD56+CD16+ subpopulation, which may be related to the insufficiency of their function due to the depletion of these cells' enzymes, perforins. It has been shown that there is a connection between reduced NK cell activity and an increased susceptibility to cancer, as well as the likelihood of cancer spread and metastasis. López-Soto and co-authors have shown that in non-metastatic pancreatic cancer, there is a suppression of NK cell function; and in metastases, there is a further increase in NK cell depletion [38].

According to Ferreira et al., the maintenance of the inflammatory reaction is possible due to a change in the signaling of the mutant *KRAS* oncogene, which contributes to the cellular organelles' destruction [39]. Activation of TLR9 in response to the free nucleic acids' presence in the interstitium and damage to mitochondria related to their dysfunction occurred with pronounced stress against the background of an increase in cortisol content during hypoxia, which accompanies pancreatic oncogenesis. Patients with non-metastatic pancreatic cancer had the most significant increase in the DAMP cytotoxic oligonucleotide fraction of mitochondrial origin in response to which specific TLR9 were actively expressed. Apparently, the previous activation of TLR4 and TLR9 receptors in the first group may contribute to further progression of the cancer process. In the examined patients with pancreatic cancer, chronic inflammation is largely due to various disorders of interconnected signaling pathways that become active in tumor cells and their microenvironment.

In the first group (non-metastatic pancreatic cancer) most patients had a pronounced increase in the IL-1β, the maturation of which is associated with the NLRP3 inflammasome pathological activation, and reflects the pronounced inflammatory process presence. In addition, Zhao et al. associates the dominant increase in IL-1β characterizes inflammasome-dependent inflammation with a chronic course or localization in the tumor microenvironment. In the second group (metastatic pancreatic cancer), some patients had an increase in TNF-α against the background of insignificant synthesis of IL-1β. It is known that in the case of TNF-α dominance, the NLRP3 inflammasome is not activated or plays a secondary role. This type of inflammation may have an antitumor, but short-term effect, but when chronic, it leads to severe multiorgan dysfunction and rapid depletion of the immune system [40].

Thus, in both groups, different dynamics of the IL-1β maturation process were found, which is manifested in an age-dependent decrease in its concentration in patients of the first group (without metastasis), and the concentration of TNF-α in this group, on the contrary, is lower in young patients and higher in older patients, which indicates the presence of more negative processes in young patients.

All deceased patients (n = 4) had a high level of TNF-α, which was associated with an increase in the level of integral cytotoxicity—the response to the content of cytotoxic serum factors in the test with *Dunaliella viridis*, an increase in cytotoxicity coefficient by 6 times.

Chronic increased IL-1β action was found against the background of reduced antigen processing ability by phagocytic neutrophils, which may enhance the pathological SASP syndrome phenotype formation and suppress apoptosis. Giroud et al. believe that senescent cells begin to actively secrete cytokines, chemokines, metalloproteinases and growth factors, enhancing the pathological SASP phenotype formation against the background of increased expression of cyclin-dependent kinase inhibitors and the accumulation of cells that are unable to divide [41].

Patients with metastatic pancreatic cancer had an increase in complement-dependent cytotoxicity, activation of T regulatory cells CD3+CD4+CD25+CD127- which contributes to the cellular anergy formation.

A very important component in the tumor microenvironment is damage-induced molecules (DAMPs), which are active regulators and are recognized by the immune system as foreign cytotoxic agents. These molecules are cellular debris that activates innate immunity and the launch of inflammatory reactions through the initiation of TLR receptor expression [18]. DAMPs have the feature of maintaining chronic inflammation in pancreatic cancer. Under the influence of these factors, the production of pro-inflammatory cytokines is stimulated, especially TNF-α, which in turn can either contribute to tumor disintegration or activate its growth. Due to the increase in the DAMP certain fractions, the immune response is suppressed and the tumor becomes less sensitive to the immune response. Chronic inflammation supports the DAMPs accumulation, the SASP phenotype formation and an overall immunosuppressive environment in the body. Against the background of tissue damage and TLR receptors activation, NLRP3 inflammasome formation may occur, which enhances the DAMPs production and stimulates the inflammatory cycle [42]. These findings also highlight the potential importance of exploring novel therapeutic strategies, including nanotechnology-based approaches for the delivery of bioactive anticancer compounds, which may offer opportunities for targeted modulation of the tumor microenvironment [43].

Patients of both groups had a wide range of antinuclear antibodies (ANA) to components of the cell nucleus: to dsDNA, TIF transcription factors, Cajal bodies, lysosomal antigens, aminoacyl-tRNA synthetases, spindle proteins, Golgi apparatus proteins, and centrosomes. Assessment of the ANA in patients with pancreatic cancer showed a higher frequency of ANA in the group of patients with metastases—59%, while among patients of group without metastasis the frequency of occurrence was 34.2%.

The detected repertoire of ANA may have a pronounced pathophysiological effect. It is known that autoantibodies to native DNA, which were found with the same frequency in both groups, can enhance apoptosis and can lead to the tumor cells destruction. But on the other hand, these autoantibodies have cross-reactions with phospholipids, which are involved in the transmission of signals that regulate cell growth and differentiation; and can contribute to the oncological process progression. Autoantibodies to transcription factors TIF1β/γ block the transcription of rRNA gene loci and are able to perform the tumor suppression function, were found with the same frequency in both groups. Loss of transcription factors function due to the presence of autoantibodies to them, changed the activation of TGFβ signaling, which activated the proliferation and growth of tumors, the development of invasion and metastasis. Patients of both groups also had autoantibodies to the histidyl-tRNA synthetase—an enzyme of the aminoacyl-tRNA synthetase class, catalyzes the addition of the amino acid histidine to the corresponding transfer

RNA, which is a critically important stage of protein biosynthesis, and ensures accurate reading of the genetic code. Patients without metastasis had the incidence only 2.6%, while patients with metastases had 14% of these ANA, which was related to the antisynthetase syndrome symptoms presence in PCS, and manifests itself in the musculoskeletal system pathology form and in the vascular ischemia form Raynaud's syndrome.

Patients with non-metastatic pancreatic cancer had antibodies to the antigens of Cajal bodies, responsible for RNA maturation and ribonucleoprotein assembly. Also, autoantibodies to glycine-tryptophan (GW-bodies) and P-bodies, (consists of transcriptional microRNAs and participates in cell cycle regulation) was detected, which are key components of membraneless cytoplasmic organelles (ribonucleoprotein granules), and that regulate the metabolism and utilization of mRNA, control the translation, stabilization and degradation of RNA molecules. In the same group, autoantibodies to the fibrillar peripheral and transmembrane proteins of the Golgi apparatus matrix, which provide the structural framework of the organelle, capture and direct transport vesicles was detected. Patients with metastatic pancreatic cancer had autoantibodies to other specificities were detected. With a frequency of up to 5%, autoantibodies to the nuclear antigen sp100, which is located in PML nuclear bodies and is a multifunctional regulatory protein. The main function of this protein is antiviral protection and regulation of gene activity. Autoantibodies to the protein HsEg5 (spindle kinesin protein), which is an important motor protein that plays a critical role in the cell division. Autoantibodies to this protein disrupt the integrity of chromosomes telomeric regions by changing the condensin protein localization. Patients with metastatic pancreatic cancer had the autoantibodies to a large structural protein (NuMA), which ensures the correct spatial arrangement of the division spindle during mitosis and participates in the formation of the nuclear envelope after the completion of mitosis. Antinuclear autoantibodies may potentially contribute to antitumor immune responses, as autoantibodies recognizing tumor-associated antigens can promote malignant cell elimination through antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity, and antibody-dependent cellular phagocytosis [44].

It is known that pancreatic tumors can evade immunogenetic control in various ways, including the secretion of immunosuppressive factors that contribute to the suppression of immunity, preventing the ability of NK cells to recognize and destroy tumor cells due to their depletion; create an immunosuppressive environment in which active cytotoxic T lymphocytes CD3+CD8+ are absent. Tumors are often able to express immune checkpoints in the form of programmed death ligands—PD-L1 and PD-L2 [45]. Emerging immunotherapeutic strategies for pancreatic cancer include immune checkpoint blockade, immunomodulation, and adoptive cell therapies such as CAR-T and CAR-NK cells, which are being actively investigated as approaches to enhance antitumor immune responses [46,47].

But the results obtained suggest that one of the main pathogenetic causes of pancreatic cancer is associated with a chronic inflammatory reaction, the dominance of immunosuppression of innate immune factors and impaired communication of adaptive immunity factors. To explain the high aggressiveness of pancreatic cancer, attention should be paid to identifying factors of the tumor microenvironment, including the altered markers of innate and adaptive immunity and stress factors identified in this study. And in the future, develop approaches to personalized correction to increase the effectiveness of complex treatment, since at present the use of surgical methods and targeted chemotherapy is not effective enough.

The work revealed insufficient antigen processing by phagocytic cells and dysfunction of mononuclear antigen-presenting cells. Liu et al. emphasize that among the mechanisms of tumor immune evasion, phagocytosis checkpoints play an important role in limiting the recognition and elimination of malignant cells by phagocytic immune cells. In particular, the interaction of tumor cell-associated MHC-I with LILRB1 receptors (Leukocyte Immunoglobulin-Like Receptor subfamily B member 1) on macrophages represents a "don't eat me" signal that can suppress tumor cell phagocytosis [48].

Chronic inflammation is an important contributor to carcinogenesis and may promote persistent epigenetic alterations that increase the risk of malignant transformation [49]. In pancreatic cancer, chronic inflammatory processes may contribute to tumor initiation and progression through complex interactions among inflammatory signaling pathways, immune cells, and the tumor microenvironment [13]. This happens because prolonged inflammation creates a microenvironment in which normal cells gradually acquire signs of malignancy. Due to the production of pro-inflammatory factors and replication disorders, additional conditions are created for the intensity of mutagenic and epigenetic disorders, tumor evasion from immune control.

Our point of view coincides with the statement of Zhang et al., that chronic inflammation leads to depletion of perforin enzymes of NK cells CD3-CD56+CD16+ and T cytotoxic cells CD3+CD8+. Tumor progression occurs

due to a change in the protective role of immune factors to an anti-inflammatory function, when the phenotype of macrophages changes from M1 to M2, Treg CD3+CD4+CD25+CD127- activation occurs and PD-L1 ligands are expressed. These mechanisms form an immunosuppressive niche in the tumor microenvironment. Different patients have various mechanisms of oncogenesis development, which make different contributions to the formation and progression of tumors [50]. Inflammation and increased infiltration of the tumor by altered immune cells are risk factors for the pancreatic cancer progression [51]. Chronic inflammatory reactions in the pancreas and the tumor process often develop not only in conditions of inflammation, but also against the background of pronounced metabolic cellular stress and SASP syndrome (Senescence-Associated Secretory Phenotype) [42].

The changes in screening tests (using the bioindicator *Dunaliella viridis* and serum electrical conductivity determination) that characterize the degree of serum factors cytotoxicity identified in the work can have both a diagnostic and prognostic role in assessing the direction, speed, and severity of the pathological processes set.

Tonello et al. are quite right in their statement that the significant heterogeneity of resistance disorders in patients is influenced by the tumor microenvironment and that unification of antitumor therapy does not currently seem possible [52]. Significant heterogeneity of the oncogenesis course in different patients causes by the individual changes in immunoresistance factors. Therefore, an individual approach is advisable, which consists in the use of personalized therapy, and makes it possible to reduce inflammation and alleviate the patient's metabolic and immune exhaustion, and thus improve clinical forecast.

5. Conclusions

Patients with non-metastatic pancreatic cancer (group 1) had a significant increase in the C3 component complement, IL-1β, IL-2, TNF-α, TLR4 and TLR9 receptors activation, and a maximum increase in the DAMPs oligonucleotide fraction. Also in the first group, an age-dependent change in the concentration of IL-1β and TNF-α was detected: young people against the background of a significant increase in the stress factor cortisol and the inflammatory process prevalence had a significant increase in the IL-1β, and in elderly patients, the inflammatory process was accompanied by activation of TNF-α production.

Patients with metastatic pancreatic cancer (group 2) had a maximum increase in the cortisol, an increase in both DAMPs cytotoxic fractions and an in the Treg cells CD3+CD4+CD25+CD127- expression, which is associated with the immunosuppressive mechanisms' activation.

In selective cohorts of both groups' patients, against the background of a pronounced violation of the studied indicators, a significant decrease in serum electrical conductivity (on average by 45% compared to the reference values) and an increase in the cytotoxicity coefficient in *Dunaliella viridis* test (by 35% compared to the reference values) were observed. These tests can be used as an express screening of the condition and prognosis of the disease course in the dynamics during treatment.

Individual changes in immunometabolic markers in non-metastatic and metastatic pancreatic cancer and age-related changes can be used to further justify personalized immunotherapy.

Author Contributions

Conceptualization, O.K.; methodology, O.K.; software, O.K.; validation, L.D.; formal analysis, O.L. and L.D.; investigation, O.L. and L.D.; data curation, R.S., O.T. and A.M.; writing—original draft preparation, O.K., O.L., L.D., R.S., O.T. and A.M.; writing—review and editing, O.K. and O.L. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

This study followed the Declaration of Helsinki principles (2013) with the Bioethics Committee of the State Institution "V.T. Zaitsev Institute of General and Emergency Surgery of the National Academy of Sciences of Ukraine" (protocol No. 4, dated 19 February 2025).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

AI Use Statement

No AI tools were used for data generation, statistical analyses, result interpretation, content fabrication, or conclusion formulation. All scientific interpretations and final revisions were independently reviewed and approved by the authors, who assumed full responsibility for the manuscript's integrity, originality, and accuracy.

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