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NON-SPECIFIC IMMUNE RESPONSE IN NSCLC PATIENTS: AN ANALYSIS OF TNF-α AND CATHELICIDIN LL-37 SERUM CONCENTRATIONS AFTER LOBECTOMY

Wydawnictwo Uniwersytetu Medycznego w Łodzi

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NIESWOISTA ODPOWIEDŹ IMMUNOLOGICZNA U PACJENTÓW Z NSCLC: ANALIZA POZIOMU TNF-α I KATELICYDYNY LL-37 W SUROWICY KRWI PO LOBEKTOMII

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WYDANIE PIERWSZE

Abstract: Major surgery induces operational stress reactions which may be associated with oversecretion of immune system biomarkers such as cytokines and antimicrobial peptides. Such disturbance of homeostasis may contribute to development of postoperative complications, such as delayed wound healing, infection or surgery-related cognitive disorders. The aim of the study was to determine the dynamics of TNF-α and cathelicidin LL-37 concentration in the serum of non-small cell lung cancer patients before and after surgery. The study group included 21 non-small cell lung cancer patients with planned tumor resection. Peripheral venous blood samples were taken from the patients before and 48 hours following lung tumor resection to assess the serum concentration of TNF-α and LL-37. The results were analyzed in terms of the extent of surgical tissue damage (depending on the selected surgical method – thoracotomy vs. videothoracoscopy) and the presence of staphylococcal skin microbiota within the operated area. There was a significant decrease in the concentration of TNF-α and a significant increase in the concentration of LL-37 after surgery. Additionally, LL-37 concentration was significantly higher in patients who underwent thoracotomy than in those subjected to videothoracoscopy. No correlation was observed between the concentrations of TNF-α and LL-37 for any of the two tested time points. In the case of a smaller incision, the operating stress may be significantly lower, which can potentially translate into an intensity of recovery after tumor resection surgery. The presence of staphylococcal skin microbiota within the operated area significantly correlated with the studied inflammatory parameters.

Keywords: tumor necrosis factor alpha, cathelicidin LL-37, non-small cell lung cancer, surgical stress, thoracic surgery

Streszczenie: Poważne zabiegi chirurgiczne wywołują reakcję stresu operacyjnego, związanego z nadmiernym wydzielaniem wykładników odpowiedzi immunologicznej, np. cytokin oraz peptydów przeciwdrobnoustrojowych. Takie zaburzenie homeostazy może przyczyniać się do rozwoju powikłań pooperacyjnych, w postaci opóźnionego gojenia się ran, infekcji czy związanych z operacją zaburzeń poznawczych. Celem badania było określenie dynamiki stężeń TNF-α i katelicydyny LL-37 w surowicy chorych na niedrobnokomórkowego raka płuca przed i po operacji. Badaną grupę stanowiło 21 pacjentów z planową resekcją niedrobnokomorkowego guza płuca. Próbki żylnej krwi obwodowej pobrano od pacjentów przed i 48 godzin po resekcji guza płuca w celu oceny stężenia wsurowicy TNF-α i LL-37. Wyniki analizowano w odniesieniu do stopnia uszkodzenia tkanki chirurgicznej (w zależności od wybranej metody chirurgicznej: torakotomia vs. wideotorakoskopia) oraz obecności gronkowcowej mikrobioty skóry operowanej okolicy. Zaobserwowano istotne obniżenie poziomu TNF-α oraz istotne zwiększenie poziomu LL-37 po operacji. Ponadto, stężenie LL-37 było istotnie wyższe u chorych operowanych torakotomią w porównaniu do chorych operowanych wideotorakoskopią. Nie zaobserwowano korelacji między poziomami TNF-α i LL-37 w obu testowanych punktach czasowych. W przypadku mniejszego nacięcia, stres operacyjny wydaje się być również mniejszy, co potencjalnie może przełożyć się na czas rekonwalescencji po operacji resekcji guza. Obecność gronkowcowej mikroflory skóry w okolicy operowanej w sposób istotny korelowała z badanymi parametrami stanu zapalnego.

Słowa kluczowe: czynnik martwicy nowotworów alfa, katelicydyna LL-37, niedrobnokomórkowy rak płuca, stres operacyjny, torakochirurgia

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1. Introduction

1.1. Lung cancer

1.1.1. Characteristics of lung cancer

Lung cancer is a primary tumor that is derived from epithelial cells. Due to the distinct biological features, clinical course, and the resultant different therapies, there are two main types of lung cancer, i.e. non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). More than 80% of all lung cancers diagnosed are NSCLC, 17% are SCLC, whereas 3% of cases are other undetermined types of lung cancer, including sarcoma and carcinoid tumors (Travis et al., 1995).

SCLC is characterized by high growth dynamics and tendency to early spread. This type of lung cancer is sensitive to cytostatic and ionizing radiation, and the treatment is usually based on the use of radiation and chemotherapy as combined therapy (Pietanza et al., 2015).

NSCLC is the most common form of lung cancer. There are three subtypes within this group, i.e., squamous cell carcinoma (SCC), adenocarcinoma (AC), and large cell carcinoma (LCC) (Rodriguez and Monaco, 2016). Squamous cell carcinoma accounts for 25-30% of all lung cancer cases. The growth of SCC is relatively slow, and its development usually begins in one of the larger bronchi. The most common type of lung cancer is AC which accounts for 40% of all lung cancer cases, both in smokers and non-smokers, in men and in women, regardless of age (Schabath and Cote, 2019; Couraud et al., 2012). As compared to other types of lung cancer, AC grows more slowly and is more often identified due to its spreading beyond the lung tissue. In contrast to AC, which grows on the periphery of the lung, LCC develops centrally in the lung and is characterized by rapid growth and dissemination. It accounts for 5-10% of all lung cancer cases and it is usually diagnosed by excluding other types of cancer. It often gives distant metastases and it is strongly associated with smoking (Brambilla et al., 2004).

1.1.2. Epidemiology of lung cancer

Worldwide, lung cancer is the leading cause of death from malignant neoplasia, in addition to prostate and colorectal cancer among men, and breast and colon cancer among women. These four types of cancer account for 43-46% of all cancer-related deaths, whereas lung cancer alone for one fourth of these cases (Siegel et al., 2019). In Poland, the National Cancer Registry data for 2012 show that lung cancer accounted for more than 14% of all 152 855 cases of malignant neoplasms among Polish patients. Lung malignancies account for about 21% of morbidities and 31% of deaths among men and 9% of morbidities and 15% of deaths among women. The risk of developing lung cancer increases with age, with a majority of lung malignancies occurring after the age of 50 years, with approximately 50% of the cases identified in both sexes after 65 years of age. The morbidity and mortality of lung cancer in men was increasing up to the beginning of the 1990s, and now the lung cancer incidence among men does not change (on average about 15 100 new cases per year), while the morbidity and mortality rates for lung cancer among women show a slightly upward trend (an increase of about 4% per year) (Jones and Baldwin, 2018; Krawczyk et al., 2015).

During the first decade of the 21st century, the 5-year survival rate among Polish patients with lung cancer increased from 11.3% to 12.6% in men, and from 16.8% to 18.5% in women (Adamek et al., 2020). In both sexes, the 5-year survival in Poland is lower than the average rate for EU countries or the USA where it reaches 19% (Siegel et al., 2019). Such low rates result, at least in part,

from the fact that more than half of lung cancer cases are diagnosed at a late stage of its development (Schabath and Cote, 2019).

Improving the treatment outcomes in lung cancer patients will depend on primary prevention (fight against smoking addiction), effective secondary prevention (screening tests, including periodic X-rays and computed tomography examinations of the chest as well as cytological examination of sputum) and the possibility of introducing personalized, targeted treatment based on genotyping and molecular markers (Hung and Chirieac, 2020; Schabath and Cote, 2019; Hung and Sholl, 2018; Jones and Baldwin, 2018; Hoffman and Sanchez, 2017; Nanavaty et al., 2014).

1.1.3. Risk factors

Lung cancer is predominantly associated with cigarette smoking, including secondhand smoke, therefore the disease is also observed in patients who have never smoked (Corrales et al., 2020; Rivera and Wakelee 2016). Despite the reduced number of smokers, 80–90% of lung cancer cases are still associated with the carcinogens released in the combustion of tobacco. They may also be caused by contact with carcinogenic chemicals and radioactive elements (like radon exposure), as well as environmental pollution, especially domestic fuel smoke and indoor air pollution. Other factors that increase the risk of lung cancer include genetic predisposition, age, previous tumors, inflammatory diseases such as asthma and sarcoidosis and also infections such as *Streptococcus pneumoniae*, *Mycobacterium tuberculosis*, *Chlamydia pneumoniae*, Human Papilloma Virus, and others (Corrales et al., 2020; Schabath and Cote, 2019; Zappa and Mousa, 2016).

1.1.4. Diagnosis and histological staging

The suspicion of lung cancer is based on medical history, physical examination and imaging studies, such as a CT scan of the chest with contrast or a PET scan which is recommended to evaluate for metastases. The final diagnosis is based on a careful microscopic examination of the material obtained from the patient (sputum, tumor biopsy) (Printz, 2020; Tsim et al., 2010; Collins et al., 2007).

Histological examination aims to differentiate between SCLC and NSCLC and to assess the severity of the disease at the time of detection. Clinical NSCLC and SCLC differentiation is based on the biological and clinical characteristics of NSCLC, such as high proliferation rates, short doubling time of tumor mass, increased tendency for early blood spread, chemosensitivity and radiosensitivity. Determination of lung cancer progression is based on the TNM classification, which takes into account the tumor size and its relation to surrounding structures (T feature), the presence of metastases in the regional thoracic lymph nodes (N feature) and metastases in distal organs (M feature) (Goldstraw and Crowley, 2006; Jones and Baldwin, 2018).

The 8th edition of the International Association for the Study of Lung Cancer (IASLC) NSCLC staging system (TNM classification) was presented in late 2016 (Goldstraw et al., 2016). It replaced the previous edition that was originally published in 2009. The 8th edition is derived from the IASLC database of 94 708 cases donated from 35 sources in 16 countries around the globe (Goldstraw et al., 2016).

The combination of T, N and M descriptors are then used to give the tumor an overall stage (I–IV). The staging of lung cancer has two objectives. Firstly, it classifies patients into groups that can

be provided with specific therapies. Secondly, staging plays an important role in defining patient groups based on expected prognosis, giving the opportunity to design clinical trials to test new therapies (Tanoue, 2008; Akhurst, 2018).

1.1.5. Surgery treatment

Generally, thoracic surgery is considered the standard care for patients with early stage lung cancer, so the accurate NSCLC staging is crucial for treatment option. For stage I and II NSCLC, the preferred primary treatment is surgical resection. The adjuvant chemotherapy for completely resected stage II NSCLC is also recommended and has proved to be helpful. In the case of patients with stage I NSCLC the use of adjuvant radiation or chemotherapy has not shown real benefits (Howington et al., 2013). However, in patients with early-stage NSCLC who do not qualify for surgery, or in the elderly, stereotactic ablative body radiotherapy (SABR) may be considered (Shinde et al., 2018).

Stage III NSCLC encompasses a heterogeneous group of patients and the treatment strategy is based on the nodal involvement, which means that patients with limited nodal involvement (N1) and more advanced nodal (N2) involvement may undergo surgical resection followed by chemotherapy and/or radiation. Patients with the most advanced nodal involvement (N3) are not generally considered to be surgical candidates (Nasim et al., 2019; Tabchi et al., 2017).

Surgery usually involves removing one lobe of the lung (lobectomy), two lobes (bilobectomy), or the entire lung (pneumonectomy). One of the most frequently used surgical access methods is classic thoracotomy which involves a several-centimeter long incision across the integument. Owing to the development of minimally invasive surgery tools, an alternative method of access is video-assisted thoracoscopic surgery (VATS) where the integument incision is significantly smaller, which means lower operational stress. Videothoracoscopy, as compared to thoracotomy, results in smaller surgical trauma, complications are much less common, and a hospitalization period is shorter (Jones and Baldwin, 2018). As shown in some studies, perioperative mortality and long-term survival following VATS lobectomy has been shown to be better than open surgery (Falcoz et al., 2016). For this reason, it is an increasingly used method for the resection of lung cancer (Reznik and Smythe, 2015; Rueth and Andrade, 2010).

1.2. Operational stress

Immune system is also involved in the reaction to injury, trauma or surgery. Most body tissues, in response to surgical manipulations, release markers of the immune response (e.g. interleukins, tumor necrosis factors, antimicrobial peptides, interferons) at the site of damage. Each surgery, injury or trauma, regardless of the body area and its extent, causes tissue damage and release of their decomposition products into the blood, which results in many local and systemic reactions (Dąbrowska and Słotwiński, 2014; Finnerty et al., 2013; Giannoudis et al., 2006; Jaffer et al., 2010). Elevated concentration of released markers in peripheral blood plasma persists after injury.

The growth rate of the immune response markers appears to be proportional to the extent of tissue damage and a type of bacteria that colonizes the damaged area. Recent research also indicates a potential impact of personality traits on the functioning of the immune system. The results of studies have shown that there is a correlation between the levels of stress perceived by patients and the concentration of proinflammatory cytokines and other wound healing factors

in their wound fluid after surgery. The body response to surgery that involves release of many factors and results in many local and general reactions is called operational stress (Finnerty et al., 2013; Giannoudis et al., 2006).

Stress is a disruption of homeostasis caused by a physical, biological or psychological stimulus. The human body is constantly exposed to various stressful situations which originate from the external or internal environment (physical, mental, metabolic stress, etc.). Body response to stress plays an important protective role, however, it may also be harmful to the host's body. The consequence of stress has an impact on the immune system resulting in disturbance of its functions. Major surgery, as the thoracic resection of neoplastic lung tumor, induces operational stress reactions associated with the modulation of the immune system active particles secretion cascade (Jaffer et al., 2010; Finnerty et al., 2013; Giannoudis et al., 2006). Such a disturbance of homeostasis may contribute to the development of postoperative complications like e.g. delayed wound healing, infection or cognitive disorders, and post-surgical delirium (Kelliher, 2011; Reznik and Smythe, 2015).

Inflammation is the main part of response following surgical manipulations. It involves upregulation of inflammatory mediators, especially cytokines which are the key modulators of inflammation. They play both inflammatory and anti-inflammatory roles since they serve as immunomodulatory elements that limit potential injury or excess inflammatory reactions. On the other hand, they may trigger either an inflammatory response or immunosuppression. The systemic response to surgery includes immunological and hematological changes, such as cytokine production, acute phase reaction, neutrophil leukocytosis, lymphocyte proliferation (Desborough, 2000; Baigrie et al., 1992).

Expression of perioperative cytokines plays an important role in postoperative organ dysfunction. A response to traumatic injury occurs immediately during surgery. Various mediators including growth factors, platelet-activating factor, reactive oxygen and eicosanoids activate various cells, such as neutrophils, endothelial cells, macrophages and lymphocytes. The cells of the liver are activated to synthesize acute phase proteins. Active particles of the immune response are secreted in the case of a surgical injury, including cytokines, such as TNF-α, as well as antimicrobial peptides, such as cathelicidin LL-37 (Chen et al., 2018a; Dąbrowska and Słotwiński, 2014; Zelová and Hošek, 2013; Jaffer et al., 2010).

1.3. Non-specific immune response

Non-specific immunity is a part of the comprehensive body defense also called the innate immunity. It includes both anatomical barriers and active defense mechanisms involving the action of chemical and biological factors, usually cooperating with specific immunity mechanisms. One of the ways how non-specific immunity works is by affecting penetrating pathogens or disordered homeostasis, through active components in the blood, lymph or secretions. The innate, non-specific response is also the first reaction of the body to immunological stress that precedes specific immune reactions. This part of the immune response includes inflammation which is a part of the complex biological response of body tissues to infection, tissue injury, trauma or surgery, neoplastic growth or immunological disorders. The inflammatory response plays an important protective role and involves immune cells, blood vessels, and various molecular mediators. The function

of inflammation is to eliminate the initial cause of cell injury, however, sometimes it can lead to tissue damage. During the inflammatory response after pathogen invasion or tissue injury, a number of reactions are initiated through cells (e.g., macrophages, endothelial cells, epithelial cells, neutrophils, dendritic cells, T-cells itself). Proinflammatory cytokines such as Il-1, TNF-α and Il-6 are released. They induce a broad spectrum of synergistic or antagonistic effects that influence the specific immune response of the body under stress (Gruys et al., 2005). The vascular system and inflammatory cells are activated. These reactions are in turn associated with production of increased amount of cytokines and other inflammatory mediators. Pro-inflammatory cytokines, nitric oxide and glucocorticoids diffuse into the extracellular fluid compartment and circulate in the blood. They activate hepatocytic receptors, trigger and modulate the systemic acute phase reaction and the synthesis of the hepatic acute phase protein begins (Gruys et al., 2005; van Miert, 1995; Heinrich et al., 1990, 1998). The acute phase response is a prominent systemic reaction of the body to local or systemic disturbances in its homeostasis caused by pathogens and tissue damage (Gruys et al., 2005).

TNF-α is one of the most important mediators in the inflammatory response. It is crucial for induction of other cytokines, such as Il-6 and Il-8 and other agents, like prostaglandins, leukotrienes, platelet-activating factor and nitric oxide. It is a protein produced predominantly by activated macrophages, T lymphocytes, monocytes but also neutrophils, keratinocytes and mast cells (Jaffer et al., 2010; Bradley, 2008). It is expressed as a proTNF on the plasma membrane where it is cleaved in the extracellular domain by the matrix metalloproteinases. It results in the release of a soluble form. The active form of TNF consists of three trimeric domains. TNF-α binds to the receptors, such as TNFR superfamily, including TNFR2 and TNFR1. Receptor binding induces activation of the cytoplasmic Janus tyrosine kinases JAK and activation of its receptors followed by recruitment of various factors beginning from transcription factors such as STAT proteins and several signaling cascades are activated. It leads to the up-regulation of transcription factors (NF-kappaB, AP-1) resulting in gene expression and secretion of various molecules, including pro-inflammatory cytokines. These diverse signaling cascades also lead to stimulation of cell proliferation and migration. Additionally, it may activate caspase cascades that result in apoptosis (Gołąb et al., 2017; Kumar et al., 2013; Miscia et al., 2002).

Not only soluble TNF, but also transmembrane TNF is involved in the inflammatory response. The transmembrane form of TNF is also able to activate TNFRs. TNF plays many important physiological and pathological roles. Expression of TNF's genes is regulated particularly by microbial endotoxin LPS (lipopolysaccharide), however, other cytokines such as Il-1 and IFN-ɣ are also able to induce TNF production and release. TNF has a pleiotropic effect. It causes tumor cell necrosis and apoptosis. TNF is an endogenous pyrogen that causes fever. It is a key mediator of both acute and chronic systematic inflammatory reactions. TNF induces its own secretion, however, it also stimulates production of other inflammatory cytokines and chemokines (Hsing and Wang, 2015). TNF is able to induce septic shock, multiple organ failure and can even lead to death (Chu, 2013). TNF-α stimulates the formation and differentiation of B, T and NK lymphocytes. It also stimulates the cytotoxicity of monocytes, macrophages and eosinophils in response to microbial invasion. It exhibits anti-tumor properties by direct action on cancer cells, inducing changes in tumor blood

vessels and modifying the immune response against cancer. All of those prove that TNF-α is one of the major cytokines involved in the immune inflammatory response (Bradley, 2008; Chu, 2013).

Cationic host defense peptides (CHDPs), also referred to as antimicrobial peptides (AMPs), are very important, conserved components of innate host defense which are up-regulated in infection and inflammation. Their cationic and amphipathic properties provide them a broad spectrum of antimicrobial activity against different species and strains of bacterial, fungal, and viral pathogens. They also prevent biofilm formation. The direct antimicrobial action is not the only, and possibly not even the primary task of antimicrobial peptides. Varied and distinctive effects of AMPs on the immune system are reported. Their additional defense roles include regulation of the inflammatory response, chemotactic activity, inducing apoptosis mechanisms (also in cancer cells), mobilization of immune cells, a direct and an indirect influence on the secretion of cytokines and chemokines, they also contribute to wound healing and angiogenesis. They are signaling molecules on the borderline of the innate and acquired immunity. They are present in storage compartments of neutrophiles and also monocytes, lymphocytes, epithelial cells and keratinocytes. AMPs are released during pathogen invasion or tissue damage. Antimicrobial peptides include defensins and cathelicidins with the only one human cathelicidin LL-37. It is a broad spectrum natural antibiotic but also a chemotactic and immunomodulatory agent (Fabisiak et al., 2016).

LL-37 is a single cathelicidin found in humans. It belongs to alfa-helical AMPs and has 37 amino acid overall length with the two leading residues being leucines after which it has taken its name. The biosynthesis of this cationic peptide in bone marrow cells is limited to the early stage of neutrophil maturation. This amphipathic peptide is synthesized in a prepropeptide form including signal peptide, propiece and C-terminal peptide. Cathelicidin LL-37 is packed as a propeptide into specific granules, especially in neutrophils, which rapidly discharge their contents extracellularly in response to inflammatory or infectious stimuli (Hsing and Wang, 2015; Zanetti, 2005).

However, the neutrophil granule storage form of cathelicidin is not an active form, therefore an additional processing step, involving proteolytic cleavage into cathelin domain and cathelicidin-derived AMP, is required (Zanetti, 2005). Cathelin-derived AMP is called LL-37. It is the predominant cleavage product of human cationic antimicrobial peptide hCAP-18 (Dürr et al., 2006; Barlow et al., 2010). Cathelicidin genes show constitutive expression in the neutrophil precursors, and/or inducible expression in various cells and tissues, such as monocytes, mast cells, NK, B, T cells and epithelial cells. Expression of cathelicidin's gene is regulated by microbial but also by inflammatory and injury stimuli (Bowdish et al., 2005; Zanetti, 2005).

After LL-37 is released from secretory granules, its antimicrobial but also stimulating functions are activated. Recruitment of inflammatory cells to the mucosa is stimulated. LL-37 activates chemotaxis of neutrophils, monocytes, and T cells via formyl peptide receptor-like 1 (FPRL-1) (Doss et al., 2009; Yang et al., 2000). On the other hand, the recruitment of neutrophils and monocytes produces an increase in LL-37 (Doss et al., 2009). It binds to Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) at an intracellular location in monocytes, leading to generation of chemokines and IL-10 (Mookherjee et al., 2009). LL-37 also down-regulates monocyte inflammatory cytokine production. It shows that LL-37 modulates further innate and adaptive immune responses. All of these mechanisms prove that cathelicidin plays an important role as a signaling molecule. LL-37 can also activate epithelial cells by interacting with EGFRs

and promote inflammatory responses of macrophages together with IL-1 (Tjabringa et al., 2003; Doss et al., 2009). All of those justify that LL-37 could be present in systemic inflammation after trauma, without evidence of bacterial focus (Hsing and Wang, 2015).

1.4. Skin microbiome

The human body is inhabited by trillions of microorganisms, collectively referred to by the term "microbiota". In contrast, the term "microbiome" refers to the sum of the collective genome of our native microbes. The human skin forms an ecosystem with a total surface area of up to 2 m^2 composed of diverse environments. From birth to death, the human body is inhabited by a vast consortium of microorganisms that include bacteria, fungi, viruses, archaea and protozoa. They are present both in and on the human skin and their diversity and abundance varies with age. The bacterial microbiota which represents the most abundant group of microorganisms inhabiting the skin is the one most thoroughly described and understood (Byrd et al., 2018; Grice and Segre, 2011; Pausan et al., 2019). It is estimated that 1 cm^3 of the skin contains up to three million bacteria (Fredricks, 2001).

The primary role of the skin, as an organ that is the interface between the body and the external environment, is its protective function. Similarly to the intestine, four components of this function may be distinguished, i.e., physical, chemical, immunological, and microbiological (Eyerich et al., 2018). The outer layer of the skin is formed by the epidermis, a barrier made up of layers of closely adherent cells that is difficult to penetrate mechanically. This layer prevents migration of particles and microorganisms to the lower layers of the skin (Bäsler et al., 2017). Substances secreted by skin cells are responsible for the chemical function. Acylglucosylceramide provides the impermeability of the epidermis to water, which protects the body from water loss. Also, the acids (fatty, lactic and uric) ensure the low pH of the skin and thus form the so-called acid layer of skin protection which has, among others, an antimicrobial effect (Miajlovic et al., 2010). The role of the skin in immune surveillance is mainly to initiate and support the local immune and inflammatory response (Eyerich et al., 2018). The skin immune response also modulates the commensal microbiota that colonizes the skin surface. Despite constant exposure to numerous microorganisms, the skin is able to distinguish between harmless commensals and harmful pathogens. Most microorganisms that reside on human skin are harmless or even beneficial to the host (Grice and Segre, 2011). The microbiota supports the barrier function of the skin and, along with other skin components, is involved in maintaining homeostasis of the human body (Eyerich et al., 2018; Grice and Segre, 2011).

As shown by molecular biology methods, primarily bacterial 16S rRNA sequencing (Kong, 2011), the bacteria that inhabit the skin belong to four different groups, i.e., Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes. Of more than 200 genera identified, three of them constitute more than 62% of all detected genera. These are *Corynebacterium* spp. (belonging to Actinobacteria), *Cutibacterium* spp. (formerly *Propionibacterium* spp. Belonging to Actinobacteria) and *Staphylococcus* spp. (belonging to Firmicutes) (Scholz and Kilian, 2016; Grice et al., 2009). The complexity, diversity, and stability of microbial populations found on the skin is variable and depends on specific anatomical location, endogenous host factors (e.g., age, sex, race, genetic variation), and environmental exogenous factors (e.g., climate, i.e., ambient humidity

or seasonal weather conditions, lifestyle, dietary and hygiene habits, use of lotions/ creams, cleansers or deodorants/ antiperspirants, types of clothing worn, antibiotics used, and hormonal status) (Fierer et al., 2010; Grice et al., 2009; Grice and Segre, 2011; Malinowska et al., 2017). Essentially, the skin is affected by coolness, dryness, acidity, as well as a lack of in nutrients. Additionally, it is highly variable in thickness, undulations, folds, distribution and density of hair follicles and glands, which results in distinct microenvironmental niches on the skin surface. Cutaneous appendages and creations, including sebaceous glands, sweat glands (eccrine and apocrine), hair or nails further influence the diversity of ecosystems on the skin surface. Studies have shown that colonization by bacteria depends on the physiology of the skin surface – the occurrence of specific bacteria is associated with moist, dry, or sebaceous microenvironments (Byrd et al., 2018; Grice and Segre, 2011). Species diversity of the skin microbiota has also been shown to be greater within and between different anatomical regions than between different individuals (Grice et al., 2009).

Species diversity of the microbiota is lowest at sebaceous surfaces, which suggests that specific organisms only tolerate conditions at these sites. At the same time, the microbiota is most stable in sebaceous regions, its structure does not change much over time. Areas with a high density of sebaceous glands, which secrete fat-rich sebum that forms a hydrophobic film on the surface, include the face, occiput, chest and back. Areas of high sebaceous gland density primarily enhance the growth of lipophilic *Cutibacterium* spp., *Malassezia* spp. and various species of *Staphylococcus* spp. (Cogen et al., 2008).

The bacteria most abundantly colonizing moist surfaces, i.e., the navel, axillary pits, elbow pits, palmar interdigital spaces, inguinal grooves, interdigital groove, knee pits, and interdigital spaces and soles of the feet, are representatives of *Staphylococcus* spp. and *Corynebacterium* spp. (Grice and Segre, 2011; Otto, 2010). Moist areas are more abundant in sweat glands. Through thermoregulatory processes, as water is evaporated from sweat, the skin is acidified, which creates conditions unfavorable for the growth of certain bacteria. In addition, sweat contains antibacterial molecules such as free fatty acids and antibacterial peptides that inhibit microbial colonization (Schröder and Harder, 2006). Metabolism of apocrine sweat by bacteria colonizing moist regions (e.g., in the axillary pits) is the reason for the characteristic sweat odor in humans (Baker, 2017). Many the so-called urea bacteria, with the participation of the enzyme urease, have the ability to utilize urea as a nitrogen source. It is supposed that bacteria colonizing the moist regions of the skin probably use urea present in sweat as a source of nitrogen (Mora and Arioli, 2014).

The most species-diverse regions of the skin surface are the dry areas, characterized by lower gland density and greater temperature fluctuations, represented by a mixture of bacteria of the genera *Cutibacterium* spp., *Corynebacterium* spp., *Staphylococcus* spp., *Streptococcus* spp. and others (with no substantial predominance of any one genus). Dry sites include limb surfaces (apart from the moist regions mentioned above), the abdomen and buttocks. Interestingly, these sites are also habitats with greater phylogenetic diversity than the intestine or mouth of the same individual (Byrd et al., 2018; Grice et al., 2009; Grice and Segre, 2011; Malinowska et al., 2017). The microbiota in wet and dry regions (high diversity) is more variable over time than the microbiota of sebaceous regions (less species-diverse) (Grice et al., 2009). However, Oh et al. (2016) have shown that

in general the microbial population on the skin does not change much over two years (which was the study period), despite continuous environmental changes (e.g., washing or use of cosmetics).

The diversity and stability of the microbiota depends not only on environmental (host-related) factors but also on interactions between the microorganisms themselves. They can interact antagonistically, e.g., competing for mutual destruction, or non-antagonistically, benefiting from each other's cooperation. Interspecies interactions both contribute to shaping of the structure of the skin microbiota and protecting the human body from colonization by pathogens and, consequently, from invasion by these potential colonizers. The mechanism has been termed "colonization resistance" (Byrd et al., 2018).

Similarly to the intestines, healthy skin is characterized by a microbiota that is highly species-diverse and stable in its structure. An imbalance in the skin's microbiological environment (dysbiosis) can lead to a reduction in the skin's barrier function. In such a situation, disease may develop as a result of random infection with a pathogenic microorganism, translocation of a particular species from another anatomical region, or excessive growth of a permanent resident population in specific parts of the skin (Belkaid and Hand, 2018; Byrd et al., 2018; Grice and Segre, 2011).

1.4.1. Staphylococcus spp. as the main representative of human skin microbiome

The best known and described genus of bacteria being representatives of the human skin microbiota is undoubtedly *Staphylococcus* spp. Staphylococci constitute one of the most numerous microbial populations of the skin (next to the genera *Cutibacterium* spp. and *Corynebacterium* spp.) and are important factors affecting health and disease of the human body. They modulate the immune function of the skin, however, they can also, as a pathogenic agent, trigger a number of diseases affecting skin tissues or any other organs. Staphylococci have been at the top of the list of microorganisms causing nosocomial infections for many years, which is mainly due to their broad distribution, widespread colonization of the human skin and mucous membranes and easy acquisition of resistance mechanisms. *Staphylococcus* genus are Gram-positive staining, spherical bacteria of 0.5–1.5 μm in diameter, occurring singly, in pairs, in tetrads, in short chains (3–4 cells), and forming characteristic clusters resembling grape clusters (hence the name staphylococcus). They are not motile, nor do they form spores. Staphylococci are facultative anaerobes and, with a few exceptions, they grow better under aerobic conditions. They represent the genus Firmicutes (Schleifer and Bell, 2009). To date, 54 species belonging to *Staphylococcus* spp. (66 including subspecies), of which about one fifth can be associated with humans as a part of their microbiota or as the etiological agents of diseases. Staphylococci are widespread microorganisms, some of them are isolated from animal samples (body cavity swabs, excretions), some can be cultured from food (meat, dairy) or inanimate environment (rock fragments, amber) (Schleifer and Bell, 2009; Švec et al., 2016). Most species isolated from humans can cause disease. It depends on the endowment of the specific strain with pathogenicity factors and the immune status of the patient. Staphylococci use a number of strategies to survive on the skin. They are halotolerant, which means that they are resistant to the high salt content of sweat and can even use urea present in sweat as a nitrogen source. They possess a number of adhesins which probably make it easier for them to attach to the skin, as well as proteases that may help release additional nutrients from the stratum corneum (Becker, 2014; Foster, 1996). Over 80% of *Staphylococcus epidermidis* strains also

produce an enzyme that esterifies fatty acids to cholesterol, which may protect them from the effects of these abundant, bactericidal lipids (Chamberlain and Brueggemann, 1997). Staphylococci prefer moist sites, and many species are often isolated from specific anatomical regions. The most numerous and common species is *S. epidermidis*. It can be isolated from the skin of all the body regions and mucous membranes. Smaller populations of *Staphylococcus hominis* and *Staphylococcus haemolyticus* are found in areas rich in sweat glands, *Staphylococcus capitis* occurs on the scalp and *Staphylococcus auricularis* in the external auditory canal. *Staphylococcus saccharolyticus* is mainly isolated from the facial skin, especially the forehead, preferring the deeper tubular sections of the skin glands. *Staphylococcus lugdunensis* is most often found in the armpit and groin area, whereas *Staphylococcus saprophyticus* in the perineal area. *Staphylococcus aureus* mostly colonizes the nasal vestibule, however, it may also occur in other moist areas on the skin (Becker, 2014; Foster, 1996).

1.4.2. Surgical site infection

The present preliminary study was focused on, among others, an analysis of the presence of staphylococcal skin microbiota at the surgical site in patients undergoing resection of NSCLC and the possible involvement of the microbiota in the so-called surgical stress reaction. This reaction leads to disturbances in skin homeostasis and thus may contribute to delayed postoperative wound healing due to the development of surgical site infections. Surgical site infection (SSI) is defined as infections that occur in the postoperative period at the site of break in the tissue continuity. They are one of the most serious postoperative complications and the most common problems in the surgical department related to surgery. According to recent studies, SSIs incidence is estimated to be 2–11% for all surgical procedures (Garner and Anderson, 2016). These include superficial infections involving the skin and subcutaneous tissue at the incision site, as well as deep infections involving the muscles and fascia and, more broadly, infections of the operated organs. The incidence of postoperative wound infection depends on a variety of factors related to both the patient and his or her disease and the medical personnel overseeing the treatment organization. The proper use of personal protective equipment and good hand hygiene of medical staff is critical to reduce the risk of transmission of pathogenic microorganisms to the susceptible patient and the environmental surfaces around the patient. SSIs are associated with increased medical costs which often require combination antibiotic therapy against multidrug-resistant microorganisms. The consequence of long-term therapy is prolonged hospital stay and increased mortality in the case of therapeutic failure. It has been shown that endogenous microorganisms of the patient are most often responsible for surgical site infections. These include bacteria that normally reside on the skin or within the operated organ – e.g., staphylococcal microbiota in the case of thoracic surgery (Stavrou and Kotzampassi, 2017). *S. aureus* and Gram-negative bacilli are major components of the bacterial microbiota of the superficial skin of hands of medical personnel, among others, which can also be a source of surgical wound infection (Boyce and Pittet, 2002). According to a study by the European Center for Disease Prevention and Control (ECDC), in recent years *S. aureus* has become the most common isolated pathogen responsible for SSIs (Zarb et al., 2012). Additionally, almost half of the cases have been shown to be caused by methicillin-resistant *S. aureus* strains (MRSA

methicillin-resistant *S. aureus*) (Anderson et al., 2015). MRSA colonization of the upper respiratory tract in surgical patients was then linked to an increased risk of SSI (Sakr et al., 2018).

It has been also proven that self-infection can occur from the reservoir of staphylococci colonizing the nasal mucosa. It has been discovered that some strains of *S. epidermidis* secrete Esp, a protease that inhibits biofilm formation and colonization by *S. aureus* in the anterior nostrils (Iwase et al., 2010). Additionally, abundance of *Corynebacterium* spp. in the nasal orifices is inversely correlated with that of *S. aureus* (Uehara, 2000), suggesting that they may also have a protective function against skin pathogens (Scharschmidt and Fischbach, 2013). In clinical infections, 80% of *S. aureus* isolates from the bloodstream correspond to isolates identified in a person's nasal cavity. Eradication of *S. aureus* in the nasal cavity of a surgical patient has been shown to significantly reduce their susceptibility to invasive infections (Byrd et al., 2018). Routine eradication poses the risk of induction of drug-resistant strains. Therefore, active screening and decolonization of the nasal passages is recommended only for those who test positive (Roth et al., 2016). In a study of 9006 patients, MRSA colonization in the anterior nasal passages was found in 4.3% of individuals. In this group, MRSA was responsible for 1.86% of SSIs as compared to 0.20% in non-colonized patients (Kalra et al., 2013). Liu et al. (2018) demonstrated the efficacy of preoperative antibiotic administration relative to placebo for inguinal hernia, breast cancer, colorectal surgery, and cesarean section. Nelson et al. (2014) showed that combination therapy is associated with a 4.14–6.87% risk of surgical site infection as compared to using the intravenous (12.76%) or oral (7.95%) route alone, with these differences being statistically significant. Thus, combined antibiotic prophylaxis (intravenous + oral) has been found to be more effective in terms of SSI prevention. Additionally, perioperative antibiotic prophylaxis has been shown not to induce bacterial drug resistance (Cohen et al., 2018). *S. aureus* is usually considered a pathogen, however, a methicillin-resistant strain of USA300 has been increasingly found to colonize moist skin sites in healthy humans (Yang et al., 2010).

The skin is the largest organ of the human body, and its protective functions are enhanced by interaction with the commensal microbiota. Together, they are a link directly connecting the body with the external environment, and at the same time, a barrier against adverse conditions of the environment. Every surgical procedure is associated with the necessity to breach this barrier, which may result in occurrence of surgical site infection. These infections are not only a medical problem but also a social one. They are associated with prolonged hospital stays, increased mortality and disfiguring scars. Considering the health outcomes and costs of treatment, research is being conducted worldwide to analyze the causality and search for methods to reduce the incidence of these infections.

2. Experimental study

2.1. The aim of the study

The study was aimed at determining the concentrations of TNF-α and LL-37 in the serum of NSCLC patients before and 48 hours after tumor resection, depending on the type of surgical access and the presence of staphylococcal skin microbiota. It was also established whether there was a correlation between the concentrations of TNF-α and LL-37 under the research conditions.

The authors tried to answer the following questions: (1) Does the size of surgical incision affect the intensity of operational stress expressed by increased concentrations of the examined factors?, and (2) Does the presence of staphylococcal skin microbiota within the operated area affect the intensity of operational stress?

2.2. Material and methods

2.2.1. Subjects

The study group included 21 patients (Tab. 1) with NSCLC hospitalized at the Department of Thoracic Surgery, General and Oncological Surgery at the Medical University of Lodz due to planned thoracic surgery – lung tumor resection. All the patients underwent standard procedures related to admission to hospital, nursing care, surgery preparation, postoperative management, and discharge. Initially, 36 patients were enrolled in the study, however 15 were disqualified, mainly due to the histopathological result indicating changes other than NSCLC.

On admission to the hospital, the patients were interviewed, and biological material was collected. Then, biological material samples were collected 48 hours after the surgery. The decisions on the type of surgery (thoracotomy or videothoracoscopy) were made by the surgeon and the anesthesiologist, based on the patients' clinical data and the availability of equipment in the operating theatre. All hospitalizations were without complications, including those related to thoracic surgery. All the patients were provided with perioperative antibiotic prophylaxis – 1000 mg of cefazolin was administered intravenously about one hour before the operation. None of the patients had an infection of the operation wound. The condition for being included into the study group was the written consent of each subject. The absolute exclusion criteria were age under 18 years, chronic inflammatory diseases, immune diseases, diabetes, renal failure, blood transfusions, intake of immunosuppressives or antihistamines, permanent medical implants, and active antibiotic therapy.

To ensure the good ethical practice of research in the project, the Bioethical Commission's approval at the Medical University of Lodz was obtained (Resolution No. RNN/146/16/KE of May 10, 2016).

Table 1. Study group characteristics.

Attribute	**TOTAL n = 21 (100%)**
Male	14 (66.67%)
Age	59.20 ±13.00
BMI	26.60 ±5.04
Surgical access	
thoracotomy	8 (38.10%)
videothoracoscopy	13 (61.90%)
Staphylococcal microbiota	
present	15 (71.43%)
absent	6 (28.57%)

2.2.2. Methods

From the patients participating in the study, the following biological material was collected: 1. peripheral venous blood to determine the concentration of the tested proteins in the serum; 2. swabs from the skin of the operated area for *Staphylococcus* spp. culture.

Peripheral venous blood samples were collected twice (before and 48 hours after the surgery) into test tubes with a clotting activator. After collection, blood was left for about 30 minutes at room temperature and centrifuged for 10 min with 1000x G overload. The obtained serum was withdrawn into clean Eppendorf tubes and stored at -20°C. Serum TNF-α concentration was determined using the High Sensitive ELISA Kit for Tumor Necrosis Factor Alpha (CCC, USA). Serum cathelicidin LL-37 concentration was determined using the ELISA Kit for Cathelicidin Antimicrobial Peptide (CCC, USA). Skin swabs from the operated area were collected before the surgery on a transport base, by rubbing a square area of 5x5 cm with a swab moistened in 0.9% NaCl. The material transported to the laboratory was inoculated on Chapman agar and incubated aerobically for 24 hours at 37°C. After macroscopic evaluation of the grown colonies, microscopic slides were prepared, and the shape and arrangement of bacterial cells were assessed. Selected strains were finally identified using BD Phoenix PX 1902 automated microbiological system (Becton, Dickinson and Company, USA).

2.2.3. Statistical analysis

The values of all the variables were presented as proportions (percentage) or medians (with range) and arithmetic means (with standard deviation). The distribution of data was checked using the Shapiro-Wilk test. The serum concentrations of the tested proteins in the samples taken before the surgery were compared with the values obtained after the surgery. The Wilcoxon signed-rank test was used to measure the significance of the difference. To compare variables between the groups, depending on the selected factor, the Mann-Whitney U test was applied. The p-value = 0.05 was considered the limit of statistical significance. The statistical analysis was performed using Statistica 13 software (StatSoft Inc., USA).

2.3. Results

Full data on the median and quartile ranges of the tested concentrations are presented in Table 2 and Table 3.

Table 2. TNF-α serum concentration values before and 48 hours after thoracic surgery (Med – median, Q_1-Q_3 – interquartile range).

	before		**48 hrs**	
	Med [pg/mL]	Q_1-Q_3 [pg/mL – pg/mL]	Med [pg/mL]	Q_1-Q_3 [pg/mL – pg/mL]
Total	7.06	4.71 – 9.41	3.74	1.64 – 4.74
Thoracotomy	7.06	5.36 – 14.65	3.12	1.57 – 4.17
Videothoracoscopy	7.04	4.23 – 9.13	4.22	2.09 – 6.51
Absent	9.16	5.31 – 24.48	3.77	2.24 – 4.20
Present	6.61	4.55 – 9.89	3.62	1.01 – 5.25

Table 3. LL-37 serum concentration values before and 48 hours after thoracic surgery (Med – median, Q_1-Q_3 – interquartile range).

	before		48 hrs	
	Med [ng/mL]	Q_1-Q_3 [ng/mL – ng/mL]	Med [ng/mL]	Q_1-Q_3 [ng/mL – ng/mL]
Total	23.18	8.92 – 31.65	42.45	26.82 – 48.81
Thoracotomy	22.38	7.27 – 40.96	45.73	40.27 – 51.52
Videothoracoscopy	23.22	10.35 – 30.65	24.32	20.68 – 39.48
Absent	23.18	11.18 – 38.69	48.07	29.40 – 61.01
Present	22.82	8.53 – 33.98	40.21	24.62 – 45.53

Preoperative TNF-α concentrations ranged from 0.07 to 39.56 pg/mL, median was 7.06 pg/mL, mean 10.19 pg/mL, standard deviation 9.45 pg/mL. Forty eight hours after the surgery, the concentration of TNF-α decreased – range 0.67–8.11 pg/mL, median 3.74 pg/mL, mean 3.52 pg/mL, standard deviation 1.58 pg/mL. Preoperative concentrations of LL-37 range 3.24–51.08 ng/mL, median 23.18 ng/mL, mean 23.89 ng/mL, standard deviation 14.85 ng/mL. Forty eight hours after the surgery, the concentration of LL-37 increased to reach values ranging from 20.32 to 64.17 ng/mL, median was 42.45 ng/mL, mean 40.13 ng/mL, standard deviation 9.76 ng/mL. For both tested proteins, significant changes of serum concentrations were observed 48 hours after the tumor resection surgery (Fig. 1). There was no statistically significant correlation between the concentrations of TNF-α and LL-37 at any of the two tested time points. There was no statistically significant correlation between the concentration of the tested proteins and the age of the patient or smoker status either.

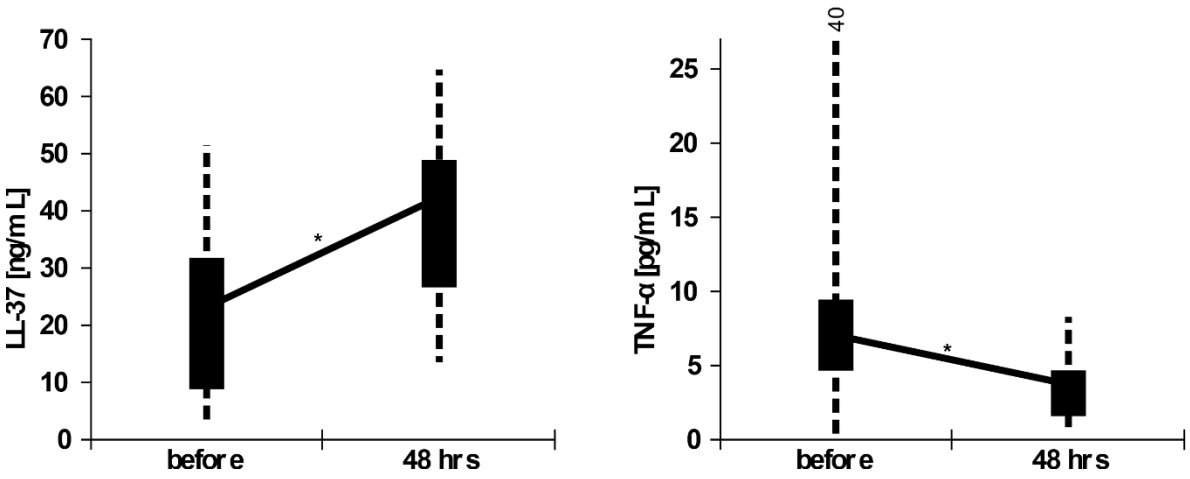

Figure 1. Value distribution of the serum concentration of the tested proteins before and 48 hours after thoracic surgery. The boxes show the interquartile range and the lines connecting the boxes show the median change, while the dashed lines present the full range, and the number above shows the outlier. The asterisk indicates the statistically significant difference ($p < 0.05$).

The statistically significant increase in the concentration of LL-37 after surgery was observed in both groups – those operated on by thoracotomy and those who underwent videothoracoscopy. However, the LL-37 concentration was significantly higher 48 hours after the surgery in the patients

operated on by thoracotomy as compared to those who were subjected to videothoracoscopy (median values of 45.32 vs. 40.13 ng/mL, respectively). In terms of the concentrations of TNF-α, a statistically significant decrease after the surgery was observed in the group of patients operated on by videothoracoscopic method. In the group of patients who underwent thoracotomy, the mean concentrations of TNF-α before and after the surgery were not significantly different (Fig. 2).

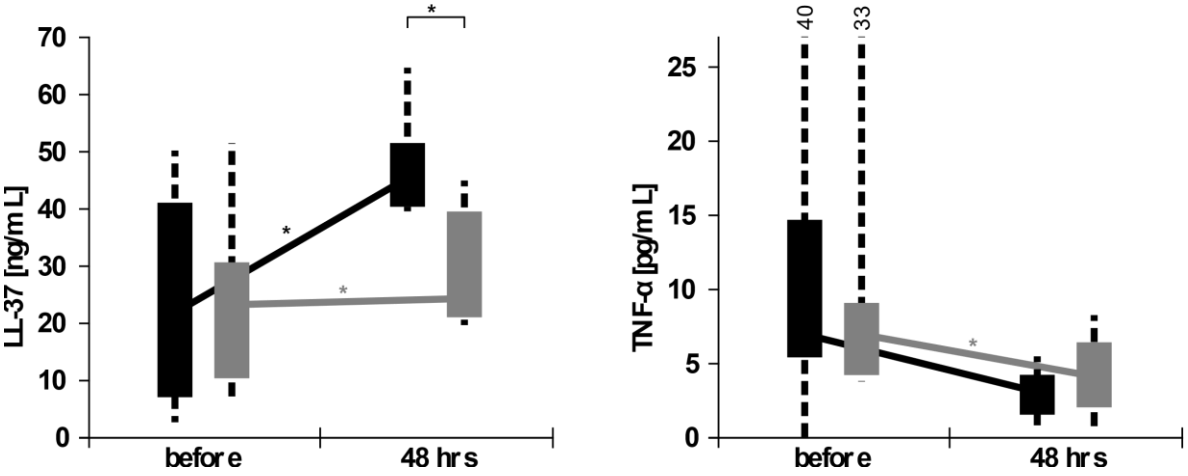

Figure 2. Value distribution of the serum concentration of the tested proteins before and 48 hours after thoracic surgery depending on the type of surgical access (black – thoracotomy, gray – videothoracoscopy). The boxes show the interquartile range and the lines connecting the boxes show the median change, while the dashed lines present the full range and the numbers above show the outlier. The asterisk indicates the statistically significant difference ($p < 0.05$).

Due to the presence of staphylococcal skin microbiota within the operated area, statistically significant changes in the concentration of the tested proteins were observed – the decrease of the TNF-α concentration and the increase in the LL-37 concentration – only in the group of patients with present staphylococcal microbiota. In the group of patients from whom staphylococci were not isolated in swabs, no statistically significant changes were observed in the concentration of the studied proteins (Fig. 3). The strains isolated from the swabs were representatives of normal staphylococcal skin microbiota.

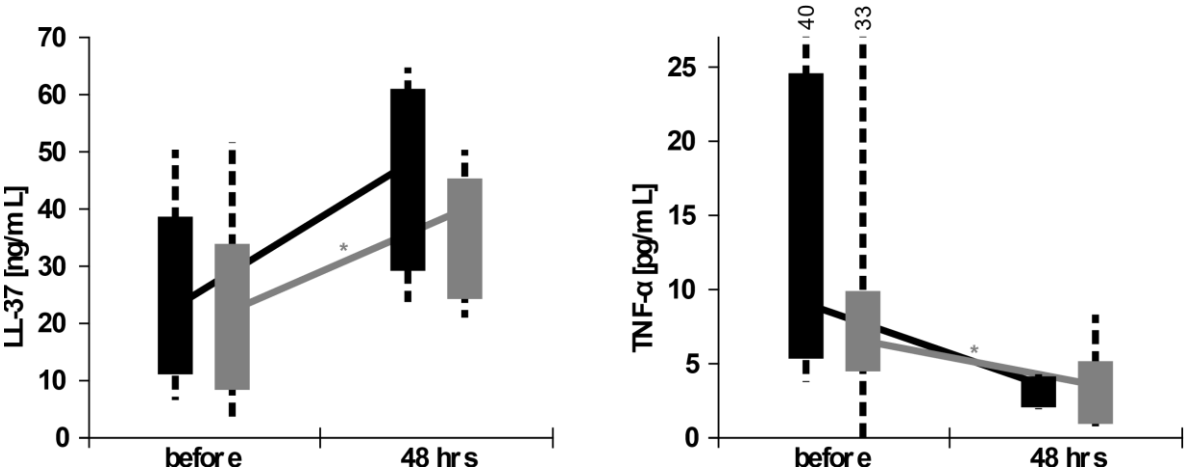

Figure 3. Value distribution of the serum concentration of the tested proteins before and 48 hours after thoracic surgery depending on the presence of staphylococcal microbiota within the operated area (black – absent, gray – present). The boxes show the interquartile range and the lines connecting the boxes show the median change, while the dashed lines show the full range and the numbers above show the outlier. The asterisk indicates the statistically significant difference ($p < 0.05$).

2.4. Discussion

Lung cancer remains the most common neoplasm in the world in terms of new cases. The diagnosis of one of its most common types – NSCLC – is often an indication for thoracic tumor resection. It is well known that during such a surgical procedure, there occurs a release of immune response particles, including TNF-α and LL-37, which participate in the so-called operational stress. LL-37 has been shown to play a role in innate antimicrobial immunity mechanisms, and TNF-α is one of the major cytokines involved in the immune response to operational stress. Therefore, it is important to understand the influence of a surgical incision in the thoracic region directly associated with the performed procedure and the presence of natural skin microbiota and its relation to the secretion of TNF-α and LL-37 in the blood serum of patients undergoing tumor resection.

Scientific publications describing the operational stress response indicate a minimal and short-term increase in TNF-α concentration in the peripheral blood following a major surgical procedure (Jones et al., 2014; Boo et al., 2007; Yim et al., 2000; Atwell et al., 1998). However, the published data are inconclusive as to the significance of the difference in the dynamics of TNF-α concentration changes depending on the type of surgical access. Some publications (Jones et al., 2014; Boo et al., 2007) show a significant change in the protein concentration after classical surgery, while others (Yim et al., 2000; Atwell et al., 1998), report no such differences. The results of our study indicate asignificant decrease in TNF-α concentration on day 2 after the major surgery. Similar results were presented by Boo et al. (2007) in a study including a group of patients after resection of the gallbladder using various methods. Such a decrease in serum TNF-α concentration may be associated with the activation of an immunologic compensatory anti-inflammatory response (Dąbrowska and Słotwiński, 2014). On the other hand, the presence of a malignant tumor may affect the secretion of cytokines. Atwell et al. (1998) observed that TNF-α concentration in lung cancer

patients was increased before the procedure and did not change significantly after surgery, as compared to the group of patients without cancer.

There are few publications about the secretion of LL-37 in relation to medical interventions. In studies conducted in patients with active pulmonary tuberculosis, a significant increase in the expression of the LL-37 gene was observed as compared to subjects with latent tuberculosis and healthy individuals (Gonzalez-Curiel et al., 2011). It was also found that the serum LL-37 concentration in people with pneumonia caused by different bacterial species was significantly higher than in healthy people. However, it should be emphasized that in this study, no statistically significant differences were found in the LL-37 concentration in patients with pneumonia caused by various etiological factors. Moreover, it was observed that in patients with an opportunistic bacterial infection, it was significantly lower than in healthy subjects (Majewski et al., 2018b). Stryjewski et al. (2007) demonstrate that the expression level of LL-37 mRNA in the skin of patients with infectious cellulitis was increased as compared to the expression in the skin of healthy volunteers. In contrast, low levels of LL-37 expression were found in biopsies of diabetic foot ulcers as compared to normal skin. Additionally, there was a lower expression of LL-37 in cultured epidermal cells from these biopsies infected with *S. aureus* as compared to cell cultures from the skin of healthy donors (Rivas-Santiago et al., 2012). In our study, we observed a significant increase in LL-37 serum concentrations after thoracic surgery. Importantly, this increase was significantly lower in the group operated on by videothoracoscopy which is associated with a smaller incision, and thus a smaller portal of entry.

Recent studies suggest that human LL-37 cathelicidin may be involved in carcinogenesis. The type of LL-37 effect, i.e., pro-carcinogenic or anti-carcinogenic, depends on the kind of tumor (Chen et al., 2018a; Yang et al., 2020). In patients with lung cancer, serum and airway surface fluid concentrations of LL-37 are elevated, which may indicate a carcinogenic effect in this type of cancer (Piktel et al., 2016; Chen et al., 2018a). Zhang et al. (2009) observed that cancer cells that stably overproduced the LL-37 precursor (hCAP-18) grew much faster. This finding suggests that LL-37 may act as a growth factor in the lung cancer (Piktel et al., 2016). LL-37 has also been shown to influence the development of lung cancer by inhibiting the biological activity of IL-32, which may be involved in the metastasis of primary lung adenocarcinoma (Hong et al., 2017; Piktel et al., 2016; von Haussen et al., 2008; Zeng et al., 2014; Choi et al., 2014).

To the best of our knowledge, there are no publications discussing the influence of staphylococcal skin microbiota on the secretion of the immune response particles after major surgery. Our results indicate a significant influence of this factor on the decrease or increase of TNF-α and LL-37 concentrations, respectively, after tumor resection in NSCLC patients with present staphylococcal microbiota within the operated area. However, further studies on a larger group are required to verify these findings and confirm or deny the influence of *Staphylococcus* spp. on the surgical stress intensity. Additionally, in a larger group of patients, it would be possible to correlate the influence of staphylococcal skin microbiota with changes in the expression level of the examined factors in relation to the surgical method used.

In this study, no correlation was observed between the concentrations of TNF-α and LL-37 at any of the two tested time points – before and 48 hours after surgery. Such results seem to coincide with the observations made by Majewski et al. (2018a), who report no correlation

between the concentrations of LL-37, TNF-α, and vitamin D in patients with tuberculosis. On the other hand, Brauncajs et al. (2018) observed a correlation between the concentrations of LL-37, TNF-α, and IFN-γ in patients with pressure ulcers treated with a low-level LLLT laser.

2.5. Study limitations

The main limitation of the study is a small study group which had an impact on the choice of the type of statistical analysis, and may have affected the power of the statistical tests. Another weak aspect is the absence of randomization, which might have influenced and biased the results. All patients of the Clinic referred to lung tumor resection surgery who agreed to participate were qualified for the study.

3. Conclusions

Our observation that LL-37 concentration was significantly higher in patients undergoing thoracotomy than in patients undergoing videothoracoscopy seems to confirm the thesis that in the case of a smaller incision, the operating stress may be significantly lower. This can potentially translate into the intensity of recovery after tumor resection surgery. Moreover, this study may also suggest that the presence of staphylococcal skin microbiota within the operated area influences the intensity of LL-37 expression which, in turn, also correlates with the selected surgical approach. However, further prospective studies on a larger group of subjects are necessary to confirm the described findings.

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