PATHOPHYSIOLOGY IMPLICATIONS OF THE THORACIC SURGICAL INTERVENTIONS

PhD Thesis

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List of full papers relating to the subject of the thesis

1.Lantos J, Németh T, Barta Zs, Szabó Zs, Paróczai D, Varga E, Hartmann P, Pathophysiological Advantages of Spontaneous Ventilation. Front.Surg. doi:10.3389/fsurg-2022.822560. (2022) **IF: 2.718**

2.Furák J, Barta Zs, **Lantos J**, Ottlakán, A, Németh T, Pécsy B, Tánczos T, Szabó Zs, Paróczai D, Better intraoperative cardiopulmonary stability and similar postoperative results of spontaneous ventilation combined with intubation than non-intubated thoracic surgery. Gen Thorac Cardiovasc Surg. 70(6):559-565. doi: 10.1007/s11748-021-01768-1. (2022) **IF: 1.517**

3.Lantos J, Furák J, Zombori-Tóth N, Zombori T, Bihari K, Varga E, Hartmann P, Changes of the T-cell composition in the thymus during the COVID-19 pandemy. [A csecsemőmirigy T-sejtjeinek összetételében létrejövő változások a COVID-19 pandémia alatt]. Orv. Hetilap 52:2059–2063. doi: 10.1556/650.2022.32664. (2022) **IF: 0,707** [Hungarian]

List of full papers not-relating to the subject of the thesis

1.Fabo Cs, Oszlanyi A, **Lantos J**, Rarosi F, Horvath T, Barta Zs, Nemeth T, Szabo Zs, Non-intubated Thoracoscopic Surgery-Tips and Tricks From Anesthesiological Aspects: A Mini Review. Front. Surg. doi: 10.3389/fsurg.2021.818456. (2022) **IF: 2.718**

2.Fabo Cs, Oszlanyi A, Barta Zs, Nemeth T, **Lantos J**, Vaida S, Szabo Zs, Anesthesiology of the spontaneous ventilation in thoracic surgery: a narrative review. AME Surgical Journal doi: 10.21037/asj-21-22. (2021)

3.Paróczai D, **Lantos J**, Barta Zs, Pécsy B, Ottlakán A, Szabó Zs, Burián K, Immunological perspectives in thoracic surgery: a narrative review on the cellular and cytokine responses induced by thoracotomy and video-assisted thoracic surgery. AME Surgical Journal doi: 10.21037/asj-21-64. (2021)

4**.Lantos J**, Nagy A, Hegedűs Z, Bihari K, Thrombolysis in case of ischemic stroke caused by aortic dissection [Thrombolysis agyi infarktust okozó aortadissectio esetén] Ideggyogy Sz. 70:1-2 pp. 69-72. doi: 10.18071/isz.70.0069. (2017) **IF: 0.252**

5.Ottlakan A, Geczi T, Pecsy B, Borda B, **Lantos J**, Lazar G, Tiszlavicz L, Klivenyi P, Furak J, Three different types of thymectomy for myasthenia gravis: Surgical and early neurological results [Myasthenia gravis miatt végzett három különböző típusú csecsemőmirigy-eltávolítás sebészeti és korai neurológiai eredményei] Magy Seb. 68(6):219-224. doi:10.1556/1046.68.2015.6.1. (2015) [Hungarian]

6.Furak J, Geczi T, Wolfard A, **Lantos J,** Lazar G, Videothoracoscopos módon, a jobb mellüreg felől elvégzett csecsemőmirigy-eltávolítás - új műtéti eljárás klinikánk gyakorlatában Magy Seb. 64(4):202-206. doi:10.1556/maseb.64.2011.4.4. (2011) [Hungarian]

7.**Lantos J**, Barta Zs, Nagy A, Vincze R, Füle K, Bihari K, A Case Study of Acute Oropharyngeal Palsy Concomitant with Diabetic Ketoacidosis, Ideggyogy Sz. 75(7-08):275-278. doi: 10.18071/isz.75.0275. (2022) **IF: 0.69**

8.Furak J, Nemeth T, **Lantos J**, Fabo Cs, Geczi T, Zombori-Toth N, Paroczai D, Szanto Z, Szabo Zs, Perioperative Systemic Inflammation in Lung Cancer Surgery, Front. Surg. doi:10.3389/fsurg.2022.883322. (2022) **IF:2,718**

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1. Introduction

All surgical intervention, including thoracic surgery causes stress, during which the homeostasis is compromised. In particular, mechanical one-lung ventilation (mOVL), the traditional method in thoracic surgery can deepen this negative effect. The surgical trauma of the intervention is accompanied by damage to the innate and the acquired immunity. The innate immunity is activated, which can induce systemic inflammatory immune responses (SIRS) and compensatory anti-inflammatory immune responses (CARS). The purpose of these immune responses is to restore the homeostasis of the immune system and start the healing process. At the same time, if the balance between these two sides is upset, it has negative consequences: if SIRS predominates, it can lead to multi-organ failure (MOF) and, in more severe cases, MODS (multi-organ dysfunction syndrome), while in the case of CARS dominance, the body's immune defense deteriorates, it becomes more susceptible to postoperative infections. The activation of the acquired immunity increases the count of the leukocytes, but also decreases the number of CD4+ and CD8+ lymphocytes, leads to a shift in the Th1/Th2 balance in favour of Th2, which can deepen the immunsuppression and the chance of infections and sepsis increases, and tumor growth and spread may increase (Dabrowska et al. 2014).

In the present work we are looking for the answer, how these harmful effects could be mitigated?

Changing the surgical method arises as a possibility, but since the vast majority of patients in thoracic surgery nowadays are operated with video-assisted thoracic surgery (VATS), which is a minimally invasive method. Several studies confirm the advantage of VATS (fewer postoperative complications, more favorable immunological changes) compared to thoracotomy (Villamizar et al. 2009, Paróczai et al. 2021).

Another option is to change the anesthetic method. Currently, mOVL and relaxation is the recommended procedure for thoracic surgery in cases of lung resection. In this method, the patient's respiratory center is turned off pharmacologically, and control is taken over by the ventilator based on pre-specified parameters. In our work, we changed this traditional method, developing the NITS (non-intubated thoracic surgery) technique.

2. Aims

1. Our first aim was to review the pathophysiologcal background of the spontaneous ventilation (SV) in thoracic surgery. We compaired the advantaged and the disadvantages of the spontaneous ventilation method between the traditional mOLV and SV methods. The non-intubated thoracic surgery (NITS) method we applied in at our clinic, can be technically divided into two parts: anesthesia and surgery. During anesthesia, the main difference of the NITS method from the relaxed-surgery method is that in NITS cases, relaxant drugs are not used, the patient is breathing spontaneously during the procedure, and tracheal intubation and mechanical ventilation are not performed (Lantos et al. 2022).

2. Subsequently, in our second study, we examined the results of NITS surgeries and the effects of combined perioperative safety procedures. Despite the many advantages of NITS, the technique still causes disputes between anesthesiologists because of potential airway loss and the complicated manner of conversion to traditional one-lung mechanical ventilation. To solve this problem, we developed a safe procedure for spontaneous ventilation thoracic surgery (SVI spontaneous ventilation with intubation). The essence of SVI is that during short-acting muscle relaxation, the patient is intubated with a double-lumen intratracheal tube, and then the surgeon applies a paravertebral/intercostal and vagus nerve block to exclude the cough reflex, and perform the VATS method. The effect of the relaxant drug ceases, and the patient breathes spontaneously through the inserted tube (Furák et al. 2022)

3. Our third study was given by the actuality of the COVID-19 pandemic. In this study, we aimed to investigate whether the SARS-CoV-2 infection itself or the vaccination against it affects the differentiation of T cells in the thymus, the histological structure of the thymus, and whether the reduction in T-cell counts observed in the blood of COVID-19-infected individuals is also observed at the tissue level in the thymus (Lantos et al. 2022)

3. Pathophysiological advantages of spontaneous ventilation

3.1. Introduction

In order to reduce the inflammatory response caused by surgical interventions, we used the NITS (non-intubated thoracic surgery) method at our clinic, which can be divided into anesthesiological and surgical parts from a technical point of view. From an anesthesiology point of view, the main difference is that in NITS, the patient does not receive muscle relaxants, is not intubated, and breathes spontaneously during the operation. From a surgical point of view, there is no significant difference compared to relaxed surgery, except that at the beginning of the surgery, the surgeon performs a paravertebral and vagus nerve block in order to eliminate the cough reflex. The two main reasons why NITS means less surgical stress for the body: 1. the patient does not receive muscle relaxants (Kiss et al. 2015) 2. the patient does not receive mechanical ventilation (Lohser et al. 2015).

3.2. Pathological changes in mOLV

The protective ventilation is a good solution for patients with normal cardiorespiratory values, but at the same time, these cardiorespiratory values are abnormal in the vast majority of thoracic surgery patients. Thus, during mechanical ventilation, if the respiratory volume is too large, volu- and barotrauma can develop, if it is too small, atelectrauma can develop (Kozian et al. 2010). Mechanical ventilation with high volume and pressure is the main cause of rupture of the alveolar wall and the alveolar-capillary membrane. Inflammatory cells (neutrophil granulocytes, macrophages, lymphocytes) flow to the site of the rupture, creating an inflammatory response (SIRS and CARS) and pulmonary edema (Lohser et al. 2015), this process is called biotrauma. Since there is no mechanical ventilation in the case of NITS, the chance of these three traumas is also much lower.

As a result of hypoxia in the lungs, so-called hypoxic vasoconstriction occurs, with this mechanism the non-ventilated lung part is excluded from the circulation, the ventilation/perfusion ratio (V/Q) deteriorates. Previous studies show that during spontaneous breathing, a V/Q ratio closer to physiological than mOLV is created (Kozian et al. 2008, Putensen et al. 1999). In the case of NITS, hypercapnia is the most common reason for conversion to mOLV, but this can be avoided in many cases with intermittent non-invasive ventilation (Kiss et al. 2015).

3.3. Cardiac and hemodynamic effects of mOLV and NITS

During mechanical ventilation, the intrathoracic pressure and lung volume are increased, which has a negative effect on the atrial filling (preload) and cardiac output. This generally affects the right ventricle only; it does not concern the left ventricle if the patient has normal myocardial function (Shekerdemian et al. 1999). With the use of the NITS method, the preload can be increased compared to relaxed-surgery cases. The difference between sOLV and mOLV can typically be observed when the thorax is just opened and the negative intrapleural pressure is lost. The development of positive intrapleural pressure during lung collapse causes hypoxic pulmonary vasoconstriction with increased pulmonary vascular resistance and diminished venous return. These changes should strain the right ventricle and cause a transient decrease in the ejection fraction of the right ventricle. In mOLV, if the patient is ventilated with positive pressure and PEEP, the hypoxic pulmonary vasoconstriction and pulmonary vascular resistance can be reduced, but in NITS, the opportunity to apply PEEP is very limited. If the surgical procedure can be interrupted for a short period to apply PEEP, it can reduce the hypoxic pulmonary vasoconstriction and pulmonary vascular resistance, but the administration of vasoconstrictive drugs is often required to stabilize cardiac output/function. After this 5–8 min period of time, when the elevated hypoxic pulmonary vasoconstriction and pulmonary vascular resistance caused by the pressure change in the thorax cavity diminished, no difference in the cardiac and hemodynamic function had been observed between the mOLV and sOLV method, based on our experience (Furák et al. 2022).

3.4. Pathophysiological effects of locoregional anesthesia in NITS

Thoracic epidural anesthesia (TEA) and paravertebral/intercostal anesthesia combined with vagus nerve block are the most frequently used locoregional anesthesia techniques in NITS. Some of the pathophysiological advantages of TEA are e.g. decreased cardiac morbidity and mortality, fewer postoperative pulmonary complications, and adequate pain management (Mineo et al. 2007). However, there are limitations to the application of TEA, from spinal cord injury to epidural bleeding/hematoma and infection (Freise et al. 2011). Studies have explored the feasibility and advantages of paravertebral/intercostal + vagus nerve block over TEA, such as lower incidences of hypotension, pulmonary and urinary complications, and vomiting and nausea, but these studies have not mentioned any effect on cardiac function and pulmonary

circulation (Davies et al.2006). In their meta-analysis, Alhayyan et al. found no significant difference in the postoperative inflammatory response between different types of anesthesia.

3.5. Immune effects of NITS and mOLV and the inflammatory response

Surgical trauma and mOLV can cause the release of damage/danger-associated molecular patterns (DAMPS). DAMPS released from damaged tissue are recognized by TLRs (Toll-like receptors). These receptors are found on the surface of macrophages and dendritic cells. Ligation of the DAMPS to receptor activates the production of the proinflammatory cytokines (IL-1, IL-6, TNF-α) These cytokines are the key factors in the communication between cells taking part in the immune response and regulation of immune activity. The normal levels of cytokines have a positive effect on the defense mechanism; however, if their release exceeds the normal level, they cause negative side effects on immune regulation, inflammation, multi-organ failure (MOF). The task of the compensatory anti-inflammatory immune response (CARS) is to maintain the homeostasis of the immune system with the help of anti-inflammatory citokines (IL-4, IL-10, IL-1Ra, TGF-β). Acquired immunity is also activated as a result of surgical trauma: leukocytosis and lymphopenia develop. A particularly important role is attributed to the shift of the Th1/Th2 balance towards Th2, because as a result of the Th2 predominance, cellular immunity decreases and immunosuppression increases, which promotes the development of postoperative infections and sepsis, as well as the growth and spread of tumors (Marik et al. 2012. , Chang et al. 2017). In several studies, Mineo et al. confirmed that after NITS surgery, inflammatory immune responses decrease and immunosuppression is also to a lesser extent.

3.6. Postoperative neuroinflammation and cognitive impairment

The immune responses mentioned above naturally also affect the central nervous system. As a result of the pro-inflammatory cytokines, the blood-brain barrier is damaged and neuroinflammation occurs, which causes the apoptosis of neurons and the reduction of the number of synapses. According to Vanderweyde et al., POCD (postoperative cognitive dysfunction) develops in 1/3 of patients over 65 years of age, while 70% of POCD patients are diagnosed with dementia 3-5 years after surgery. According to WHO data, nowadays approx. 55 million people suffer from dementia and approx. 10 million new cases are diagnosed, and with the current trend, the number of dementia patients is estimated at 139 million by 2050. In the background of dementia, the cases are approx. 70% have Alzheimer's disease, the clinical

appearance of which can be advanced by years due to the stress of surgery. The reduced inflammatory and immune changes after NITS suggest that neuroinflammation and cognitive impairment are also reduced after NITS compared to relaxed-surgery. With the help of NITS, the risk of POCD and dementia can be reduced.

3.7. Effects of NITS on cancer

The tumor itself and the surgery to remove it upset the Th1/Th2 balance already mentioned above. As a result of surgical stress, Th2 predominates, which promotes tumor growth and spread. At the same time, if Th1 predominates with the increase of cellular immunity, it promotes the destruction of the tumor. During the application of NITS, the Th1/Th2 balance can be maintained better, it is less shifted towards Th2, thus the ability of the immune system to destroy/inhibit tumors can remain more active (Chang 2017). Today, the most promising cancer treatment is immunotherapy, which is more effective the more physiological the patient's immune status. Since immunosuppression is less with NITS compared to mOLV, this suggests that postoperative immunotherapy after NITS should be more effective. Based on these considerations, we can expect better long-term survival data in tumor surgery performed using the NITS method.

3.8. Effects of relaxant drugs on immune function

The indirect effect of relaxation on the immune response in mOLV is detailed above, but the relaxant agents also affect the direct release of cytokines from macrophages. One experimental study implicated the presence of acetylcholine (ACh) and α7ACh receptors on blood mononuclear cells and the cholinergic anti-inflammatory pathway, showing that ACh significantly reduced the release of pro-inflammatory cytokines from human macrophages in culture. The drugs used to induce relaxation in mOLV block the neuromuscular junction by binding to the ACh receptors in combination with ACh. In theory, relaxants could block the ACh activity on macrophages; however, to our knowledge, whether relaxant drugs bind to ACh receptors on macrophages has not been demonstrated. It is likely that α7ACh receptors can be found on both the postsynaptic muscle membranes and the surfaces of macrophages. (Benfante et al. 2021). Therefore, relaxation has a double effect on the immune system: through mOLV-induced cytokine release and through the release of cytokines from macrophages. Both of these mechanisms can be avoided by performing NITS.

3.9. The summary advantages of NITS

- minor volu-, baro-, atelecto- and biotrauma

- closer to physiological ventilation/perfusion ratio (V/Q)

- better right atrial filling (preload)

- less SIRS and CARS, less immunosuppression

- less neuroinflammation, less postoperative cognitive deficit, dementia?

- more quality years of life?

- decrease in tumor progression, increase in tumor regression?

- more effective response to immunotherapy? better long-term survival?

4. Comparison of cardiopulmonary and postoperative results of spontaneous breathing combined with intubation (SVI) with NITS

4.1. Introduction

At our clinic, we created a new method of spontaneous ventilation with a safe airway, which unites the uniportal video-assisted thoracic surgery (VATS) technique with minimal duration of relaxation, intubation, and mechanical one-lung ventilation followed by spontaneous ventilation of the patient (SVI). In this study, we compared the intraoperative feasibility and postoperative results of NITS lobectomies to those of lobectomies performed using the SVI method. In our work, we analyzed 38 NITS and 38 SVI VATS lobectomy data.

4.2. Method of SVI

At the beginning of the procedure, a short-acting relaxant drug (mivacurium) was administered, and the patients were intubated with a double-lumen intratracheal tube. After that, uniportal VATS incision was performed with local anesthesia. To avoid coughing triggered by the intratracheal tube, vagus and paravertebral nerve blockades were performed. Bupivacaine (4–5 mL) was administered near the intercostal nerve between the second and fifth intercostal spaces close to the spine (paravertebral blockade) and near the vagus nerve. Later, the patients arose from the initial relaxation and could breathe spontaneously. The bispectral index (BIS; Medtronic Vista) was controlled with propofol to maintain a BIS of 40–60. Due to the vagal

nerve blockade, the intratracheal tube did not trigger coughing, and the movements of the thoracic cavity and mediastinum do not disturb the course of surgery. Meanwhile, a safe airway was ensured due to the double-lumen intratracheal tube during the entire procedure, all possible complications were managed easily, and conversion to mechanical one-lung ventilation is fast and safe. Anaesthesiologic indications for conversion to relaxation and mOLV are hypoxemia and hypercapnia. According to experience, the hypercapnia during surgery does not cause problems, as long as the $PaCO_2$ is less than 70 mm Hg (permissive hypercapnia) or the pH does not fall below 7.15. We considered a body mass index (BMI) of more than 30 as the only exclusion criterion for SVI cases (Furák et al. 2022).

4.3. Patients

In the case of NITS VATS operations, we examined the data of 38 patients, 22 women and 16 men, the average age of the patients was 64.9 years, their average BMI was 25, while the average FEV1 was 90.4%. We also included 38 patients in the SVI VATS group, 18 women and 20 men, their average age was 65.4 years, their average BMI was 26.7, and their average FEV1 was 87.1%. The oncological aspects of selection are similar to the criteria applied in the SVI group, and it is based on the current recommended guideline (the diameter of the tumor is less than 7 cm; the lymph node stage is cN0 or cN1) (Yan et al. 2014).

4.4. Intra- and perioperative results

Significantly lower lowest systolic and diastolic blood pressures were found in the NITS group versus the SVI group (systolic: 83.1 vs 132.3 mmHg) (p=0.001); diastolic: 47.8 vs. 73.4 mmHg) (p=0.0001). The lowest oxygen saturation was signifcantly lower in the NITS group than in the SVI group (90.3% vs 94.9%) (p=0.026). The highest pCO_2 level was signifcantly higher in the NITS group than in the SVI group (62.5 vs 54.8 kPa) (p=0.009). There were no signifcant differences in the other parameters measured by anesthesia. In the NITS group, the conversion rate to relaxation, intubation, and mechanical one-lung ventilation in the NITS group was 5.2% (n=2). In two additional patients, because of hypoxia, both lungs were temporarily ventilated (reinflation) one or more times to reach the normal oxygen saturation level. This means that in these four cases, the normal NITS method was not successful. In the SVI group, this both lung temporary ventilation method to elevate the saturation was not used. In the SVI group, there were ten cases (26.3%) of spontaneous ventilation via the double-lumen tube with PEEP (3–5 cmH2O) and FIO_2 (40–100%) was not sufficient to maintain acceptable gas exchange parameters. Five of them (13.1%) were relaxed and mechanical one-lung

ventilation was applied, and an additional 5 of the 38 cases (13.1%) received PSV to the dependent lung to support spontaneous ventilation, but they were not relaxed. The reason for conversions was gas exchange insufficiency. From this point of view, the failure rate of spontaneous ventilation method due to hypoxia was 10.5% (4/38) in the NITS group versus 13.1% (5/38) in the SVI group (p=0.724).

4.5. Histological results

Primer lung cancer was removed in 33 cases in the NITS group versus 26 cases in the SVI group. Two metastasectomies were performed in the NITS group versus 6 in the SVI group. Benign lesions were removed in 3 and 6 cases in the NITS and SVI groups, respectively. We removed a mean 11.2 mediastinal lymph nodes in the NITS group; versus 14.7 in the SVI group (p=0.109). In both groups, 10.5% of the removed lymph nodes were metastatic (p=1.000).

4.6. The summary advantages of SVI

- safe airway
- fast and safe option in case of indication of conversion to mOLV
- PSV (pressure support ventilation) and PEEP (positive end-expiratory pressure)

  possibility of application
- significantly higher systolic and diastolic blood pressure values during surgery
- more stable cardiopulmonary condition during surgery
- significantly higher oxygen saturation during surgery
- significantly less hypercapnia during surgery
- better gas exchange during surgery
- shorter surgery and drainage time

5. The COVID-19 pandemic and the thymus

5.1. Introduction

The third major part of our thesis is given by the actuality of the COVID-19 (coronavirus disease-19) pandemic. In our study, we aimed to investigate whether the SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) infection itself or the vaccination against it affects the differentiation of T cells in the thymus, the histological structure of the thymus, and whether the reduction in T-cell counts observed in the blood of COVID-19-infected individuals is also observed at the tissue level in the thymus.

The main function of the thymus is to differentiate T cells, i.e., to create T cells that have a T cell receptor but are not autoreactive. Among the T cells entering the bloodstream, we distinguish cells with different functions:

The CD4+ T cells are called T helper cells, which are activated by a foreign antigen and begin to divide vigorously. Through cytokines, they promote the maturation of B cells into plasma and memory cells and the activation of cytotoxic T cells and macrophages.

The CD8+ T cells are known as cytotoxic or killer T cells, which recognize and kill cells that carry a foreign antigen, thus playing a central role in the defense against viruses and tumors.
Regulatory T cells (Treg) that develop in the thymus express the transcription factor FOXP3, and their main function is to monitor immunotolerance, thereby providing protection against autoimmune mechanisms.
The maturation of natural killer (NK) cells also occurs in the thymus, which cells are the link between the innate and acquired immune systems. The CD25 marker was previously thought to be found only on natural killer cells, but it has now been shown to be present on all activated T lymphocytes and to play a role in regulating the cellular immune response. High CD25 levels result in reduced immunotolerance to self-antigens, which is why high CD25 levels are characteristic of autoimmune diseases, including myasthenia gravis (Miller et al.2002).
With advancing age, the thymus atrophies, which leads to immune aging in the body, resulting in a decrease in the number of T and B cells as well as the NK cells. It is therefore logical that immunity to new antigens, infectious agents and vaccination declines with age, and this explains why COVID-19 infection causes the most severe morbidity and highest mortality in the elderly population and in patients with primary immunodeficiency (Gong et al. 2020, Maródi et al. 2022).

The SARS-CoV-2 virus reduces the antigen presentation to cytotoxic T cells, thereby reducing the antiviral effect of T cells (Váradi et al 2020). A significant reduction in the number of CD8+ T cells, memory CD4+ T cells and regulatory T cells was observed in the blood of COVID-19-infected patients, with concomitant reductions in CD4+ and CD8+ T cell counts in the lymph nodes and spleen of these patients (Liu, Paces, Zheng et al. 2020). Although it has been suggested in the literature that the worsening immunological status in coronavirus infection may be due to lymph node and spleen atrophy and reduced lymphocyte count in the blood of patients, to our knowledge, no comparative histological examination of the thymus has been performed to date. Autopsies of those who died from coronavirus infection showed marked atrophy in both the spleen and the hilar lymph nodes (Falasca et al. 2020).

5.2. Patient population

Data from a total of 55 patients who underwent thymectomy were analyzed to create three groups: 1. The pre-COVID-19 (PC-) group includes 22 patients; 12 women and 10 men, with a mean age of 29.27 (16-50) years, who underwent thymectomy between 2008 and 2013, before the COVID-19 pandemic. 2. No-COVID-19 (NC-) group: although 20 patients (11 women and 9 men, mean age 45.75 (19-75 years) in this group underwent thymectomy between 2020 and 2021, during the COVID-19 pandemic, these participants did not have confirmed COVID-19 infection and were not vaccinated either 3. Vaccinated or Infected COVID-19 (VIC-) group includes 13 patients (4 women and 9 men, mean age 49.76 (22-74 years), who also had a thymectomy in 2020-2021, during the COVID-19 pandemic, but either had a confirmed COVID-19 infection or received a COVID-19 vaccine.

5.3. Results

5.3.1. Histological findings

In the PC group, histopathological examination confirmed thymic hyperplasia (Figure 9.) in 16 cases and persistent thymus in 6 cases. The indication for thymectomy in 12 patients was myasthenia gravis.In the NC group 9 cases of thymic hyperplasia, 5 cases of persistent thymus, 4 cases of thymoma (2 type A thymomas, 1 type B1 thymoma and 1 type B2 thymoma), 1 patient with thymic cyst, 1 patient with thymic lipoma were confirmed. Thymectomy was performed in 13 cases due to myasthenia gravis. In the VIC group, 4 cases of thymic hyperplasia, 3 cases of persistent thymus, 5 cases of thymoma (1 type B1 thymoma, 2 type B3 thymomas and 2 type AB thymomas) and 1 patient with thymic cyst were diagnosed. In this

group, 4 patients had myasthenia gravis. In the cases of thymomas, immunohistochemistry was performed on the part of the thymus not involved by the tumor.

5.3.2. Immunhistochemical results

There was a significantly lower CD4 reaction in the VIC group than in the PC group (p=0.007) and in the VIC group compared to the NC group (p=0.041), but there was no significant difference between the P and the NC groups (p=0.542). There were no significant differences in the CD8 staining between the PC, NC and VIC groups of patients: (p=0.246), but the CD8+ cell staining was higher in samples from the COVID-19 era, as compared to the pre-pandemic period. The FOXP3 staining was significantly lower in the VIC group compared to the PC group of patients (p=0.001) and in the NC compared to the PC group of patients (p=0.001), but there was no significant difference in the FOXP3 expression between the thymic samples of NC and VIC groups (p=0.568). There were no significant differences between the PC, NC and VIC groups regarding the CD25 staining of thymic samples.

5.4. Conclusion

In our work, we found that the SARS-CoV-2 infection or the vaccination against it affects both the cellular composition of the thymus and the humoral immune response. Significantly lower CD4+ level was measured in the VIC group compared to the PC and NC groups, suggesting that the number of T helper was significantly lower in the thymus of patients who have been vaccinated or have been confirmed to have COVID-19 infection. Due to the lower T helper cell count, the activation of cytotoxic T cells and macrophages, and the maturation of B lymphocytes into plasma and memory cells are reduced. In summary, the body's ability to recognize and destroy foreign antigens deteriorates. For the markers CD8+ and CD25+, no significant differences were found between the three groups, so our study did not confirm a significant decrease in the number of activated T-lymphocytes in the samples of vaccinated or infected patients. Significantly lower levels of FOXP3 were confirmed in the VIC group compared to the PC group (p=0.001) and in the NC group compared to the PC group (p=0.001), however, no significant difference was observed between the VIC and NC groups (p = 0.568). The significantly lower FOXP3 levels observed in the VIC group compared to the PC group suggest a reduction in the number of regulatory T cells (Treg) in patients who have been vaccinated or have been confirmed to have COVID-19 infection, which leads to a deterioration

in immune tolerance, making it easier to develop autoimmune diseases such as myasthenia gravis. For FOXP3, the significant difference observed between the NC and PC groups may be due to the high number of asymptomatic COVID-19 infections. A report - published in December 2021-, found the rate of asymptomatic coronavirus infection to be 40.5%, based on the meta-analysis of the results of 95 studies. (Oran et al. 2021). In our case, this high percentage of asymptomatic infections may underlie the fact that there are patients in the NC group who have not been vaccinated nor have had a confirmed infection, however, their results show the differences observed in the VIC group.

In our study, we found with immunohistochemical examination that there is no significant difference between the three groups in the case of CD25, however, in the case of FOXP3, we observed a marked or significant decrease in patients detected during the COVID-19 pandemy. Comparing the thymic immunohistochemical results of the VIC group with the changes in the serum of COVID-19 infected patients reported in the literature, the following results were obtained: there is also a decrease in the number of T helper, Treg lymphocytes in the thymus, as in the serum of COVID-19 infected people (Liu, Paces, Zheng et al. 2020), but no decrease in the number of the killer T cells was detected in our study. During autopsies of COVID-19 infected patients, the severe atrophy, observed in the lymph nodes and the spleen (Falasca et al. 2020), was not confirmed for the thymus, which may also be due to the mild course of the COVID-19 disease among the patients included in our study. As the autopsy results do not include thymic data, we are unable to compare the data and determine whether fatal COVID-19 infection causes atrophy in the thymus as well.

6. Summary of new findings

1. In our first review study, we summarized the SIRS theory in thoracic surgical point of view and compared spontaneous ventilation with mOVL. We found, that the performing NITS with spontaneous ventilation can prevent or reduce volu- baro-, and atelectotrauma in the alveoli that is caused by mOLV in relaxed-surgery cases. Due to the reduced pro-inflammatory response and release of fewer cytokines, NITS also can prevent or reduce biotrauma. The reduced pro-inflammatory response and cytokine release can reduce neuroinflammation and cognitive dysfunction and can also reduce the spread of cancer. Currently, the most promising treatment for cancer is immunotherapy. According to the concept of immunotherapy, the more physiological the postoperative immune system is, the more effective is the anticancer function. The immunosuppression is less after sOLV than after mOLV, suggesting that after NITS the

postoperative immunotherapy should be more effective. During surgery with spontaneous ventilation, the V/Q match is better, which results in better oxygenation and cardiac output, as compared to relaxed-surgery cases. The abovementioned pathophysiological advantages are the basis of the clinical observation that there are fewer complications after NITS lung surgery than in relaxant-surgery cases. Direct clinical and pathophysiological arguments (reduced inflammatory response, limited change in the number of leucocytes, and fewer postoperative morbidities) can support our theory that the NITS procedure is more physiological than mOLV.

2. In our second study, we created a new method of spontaneous ventilation with a safe airway. With the help of SVI, we can preserve most of the advantages of spontaneous ventilation, but all this with a safed airway. In the SVI cases we found better cardiopulmonary stability. That means that cardiac function is more stable during the SVI procedure than during the NITS procedure. The mean lowest oxygen saturation was significantly higher in the SVI group than in the NITS group, while the highest mean pCO_2 level was significantly lower in the SVI group than in the NITS group. From these data, it can be concluded that the SVI procedure provides better gas exchange during spontaneous ventilation due to the PEEP of 3–5 cmH_2O and the PSV. With this procedure, better oxygenation and better CO_2 changes cause smaller hypoxic pulmonary vasoconstriction, thus it decreases less the blood pressure.
3. In our third study, the results confirmed the previous hypothesis that significant immunological changes are induced by the SARS-Cov-2 virus. However, our study group was the first to demonstrate this for the thymus.. In the thymus, the T helper cell function decreases, resulting in a reduction in immune defense. The lower T helper cell count results in reduced activation of cytotoxic T cells and macrophages, and in a reduced maturation of B lymphocytes into plasma and memory cells, that is, the body's ability to recognize and destroy a foreign antigen is impaired. We found lower FOXP3 values in both patient groups of the COVID-19 pandemic period compared to data from patients of the pre-COVID-19 pandemic period, while comparing the values of the two COVID-19 pandemic period patient groups yielded no differences between the infected or vaccinated and the uninfected and unvaccinated patients, which may be due to the high number of the asymptomatic coronavirus infections.

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