PATHOPHYSIOLOGY IMPLICATIONS OF THE THORACIC SURGICAL INTERVENTIONS

PhD Thesis

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CONTENTS

List of abbreviations

ACh	acetylcholine
AIDS	acquired immune deficiency syndrome
ALI	acut lung injury
APC	antigen-presenting cell
BBB	blood-brain barrier
B cell	type of lymphocyte, developed in bone marrow
BIS	bispectral index
BMI	body mass index
BP	blood pressure
Ca	calcium
CARS	compensatory anti-inflammatory immune responses
CCI	Carlson Comorbidity Index
CD	cluster of differentiation
CO_2	carbon dioxide
COVID–19	coronavirus disease 2019
CRP	C-reactive protein
CT	computed tomography
DAMPS	damage/danger-associated molecular patterns
FEV1	forced expiratory volume in 1 sec
FIO_2	fraction of inspired oxygen
FOXP3	forkhead box P3
GSK-3	glycogen synthase kinase-e
HPV	hypoxic pulmonary vasoconstriction
IgG	immunoglobulin G
IL	interleukin
iNOS	inducible nitric oxide synthase
INR	international normalised ratio
MODS	multiple organ dysfunction syndrome
MOF	multi-organ failure

mOLV	mechanical one-lung ventilation
NC group	No-COVID-19 group
NITS	non-intubated thoracic surgery
NK	natural killer cell
NO	nitric oxide
NOD	nucleotide-binding oligomerization domain-containing protein
O_2	oxygen
PAL	prolonged air leak
PAMPS	pathogen-associated molecular patterns
PC group	pre-COVID-19 group
pCO_2	partial pressure of carbon dioxide
PEEP	positive end-expiratory pressure
pH	potential of hydrogen
POCD	postoperative cognitive dysfunction
PVR	pulmonary vascular resistance
PSV	pressure support ventilation
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SIRS	systemic inflammatory immune responses
sOLV	spontaneous one-lung ventilation
SpO_2	saturation of oxygen
RT-PCR	reverse transcription-polymerase chain reaction
SV	spontaneous ventilation
SVI	spontaneous ventilation with intubation
T cell	T lymphocyta or thymocyta
TEA	thoracic epidural anesthesia
TGFβ	transforming growth factor β
Th cell	T helper cell
TLRs	Toll-like receptors
TMA	tissue microarray
TNF	tumor necrosis factor

Treg	regulatory T cell
VATS	video-assisted thoracic surgery
VIC group	vaccinated or infected COVID-19 group
V/Q ratio	ventilation/perfusion ratio
WHO	World Health Organization

List of original papers

List of full papers relating to the subject of the thesis

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**

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**

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Cumulative IF: 11,32

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 due to the development of pathogen-associated molecular patterns (PAMPS) and the damage/danger-associated molecular patterns (DAMPS) in the early postoperative period, which can induce systemic inflammatory immune responses (SIRS). SIRS is advantageous for the body as it reduces tissue damage, removes residues of dead cells and starts the healing process. If SIRS becomes severe or prolonged, it can lead to multi-organ failure (MOF) or, in more severe cases, even multiple organ dysfunction syndrome (MODS). Compensatory anti-inflammatory immune responses (CARS) is activated to reduce the severity and duration of SIRS. The adverse effect of CARS can be the increased immunsuppression, predisposing patient to sepsis, secondary infections or late MODS. 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. As a result of these immune reactions, the body's ability to defend itself deteriorates, the risk of acute lung injury (ALI), MOF, MODS and infections increases, the hospital time and the associated costs also increase [1].

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. VATS has fewer postoperative complications than thoracotomy [2]. Many retrospective and prospective studies approve that VATS can be associated with less decreased natural killer (NK) and T cell numbers, phagocyte and oxidative acticivity and reduced levels of cytokines, compared to thoracotomy [3].

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.

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 [4].
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 [5].
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 [6].

2. PATHOPHYSIOLOGICAL ADVANTAGES OF SPONTANEOUS VENTILATION

2.1. Background

Surgical procedures, including thoracic surgery, cause stress, which can induce inflammatory responses and reduce the function of the immune system [7]. Ventilation applied during surgery can deepen this negative effect [8]. These alterations influence the healing of patients after surgery, and more-intensive and longer trauma results in greater surgical stress and an increased inflammatory response [9]. To reduce the stress to the patient during thoracic surgery, minimally invasive procedures can be applied. Video-assisted thoracoscopic surgery (VATS) has fewer postoperative complications than thoracotomy [2], and the inflammatory response is reduced after VATS compared to open surgery [3, 10]. Following the innovation and acceptance of VATS, non-intubated thoracic surgery (NITS) was developed to further reduce surgical stress in thoracic surgical procedures [11]. It has been shown that the inflammatory response after NITS is lower than that in intubated- and relaxed-surgery cases [12], and it is suggested that NITS might be a least minimally invasive thoracic surgical procedure [13]. NITS 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. The surgical part of NITS is the same as that in relaxed-surgery cases, but in the internal paravertebral/intercostal + vagus nerve block method, the surgeon administers the local anesthesia (Figures 1, 2). Two key advantages of NITS that can help reduce surgical stress and inflammatory responses are the non-use of muscle relaxant drugs [14] and the absence of mechanical one-lung ventilation (mOLV) [15]. The use of spontaneous ventilation instead of mOLV results in a lower inflammatory response and immune alteration and induces a different pathophysiological state of the cardiorespiratory system.

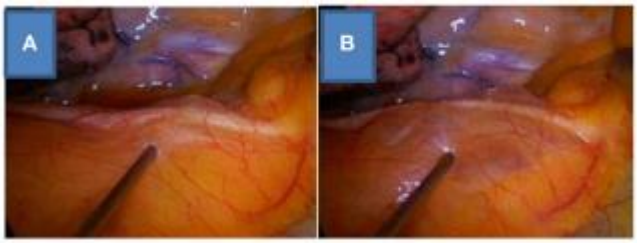

Figure 1. Vagus nerve block on the right side, before (A) and after (B) the local anesthesia [4].

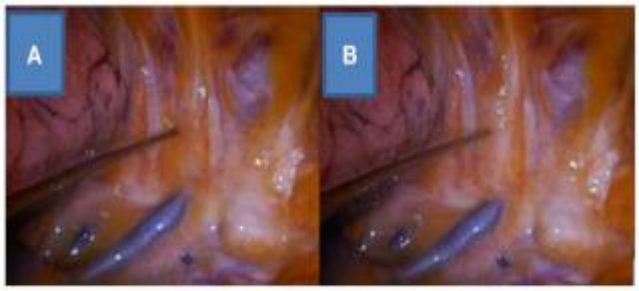

Figure 2. Internal paravertebral/intercostal nerve block on the right side before (A), and after (B) the local anesthesia [4].

2.2. Effects of mOLV

Mechanical one-lung ventilation and relaxation is currently the recommended procedure for thoracic surgery in cases of lung resection. It is an obligatory process and provides a good view of the patient's anatomy; however, it has negative effects that must be taken into account. The terminology of one-lung ventilation was created in the pre-NITS period, and it is currently classified into two subclasses: mOLV mechanical one-lung ventilation (previously called one-lung ventilation) and spontaneous one-lung ventilation (sOLV). There are many pathophysiological differences between the two methods. In mOLV, maintaining the oxygen level within or close to the physiological range is achieved by increasing the oxygen level or positive end-expiratory pressure (PEEP) or using ventilation with positive pressure (PSV). However, hypoxia develops in 30–90% of patients during mOLV. The parameter settings on the ventilator depend on the experience of the anesthesiologist. The lung alveoli can be damaged if these parameters are non-physiological or intolerable for the lung parenchyma, mainly at the capillary membrane of the alveoli and especially at the endothelial glycocalyx. These changes are the pathophysiological basis of acute lung injury (ALI) caused by ventilation [16]. To reduce or avoid these complications, protective ventilation is recommended, which include keeping the tidal volume to 4– 5 ml/kg, ensuring a PEEP of 5–10 cmH_2O, performing a lung recruitment maneuver, and using volatile anesthesia [17]. The application of protective ventilation is easy or manageable in patients with normal lung parenchyma and cardiac function; however, if the patient has obstructive pulmonary disease with pre-existing pulmonary hypertension, protective ventilation can be difficult. Even a slight change in the lung volume in mOLV can cause hypoxia, hemodynamic imbalance, and elevated pulmonary vascular resistance with reduced cardiac output; the correction of the oxygenation requires

ventilation with a higher volume and pressure, which can cause injury to the alveoli [18]. Clinically, mOLV is similar to the ventilation performed in post-pneumonectomy cases; in a clinical study, an increase of 1 ml/kg of tidal volume in post-pneumonectomy patients was associated with approximately four times more chance of abnormal pathophysiological changes in the lung [19]. It can be concluded that the ventilator setting during mOLV, which is used mainly for patients with underlying lung parenchymal disease with low pulmonary capacity, is vulnerable to changes, and a large tidal volume can cause end-inspiratory lung overdistension (volutrauma), which has a high risk of postoperative pulmonary complications [20]. Moreover, atelectasis (atelectrauma) may develop in cases of low-volume ventilation, and the abovementioned lung injury (volutrauma) can develop during the correction of the atelectasis. The use of NITS in these patients may be beneficial for preventing these pathophysiological changes.

2.3. Pathological changes in mOLV

High-volume ventilation and high-pressure ventilation are the main risk factors for damage to the alveoli during mOLV. Elastic and collagen fibers determine the elasticity of the lungs, and the alveolar wall with the alveolar-capillary membrane and endothelial glycocalyx can be ruptured in cases with greater than normal stretch. Lung parenchyma injury can result in cytokine release, recruitment of inflammatory cells (neutrophils, macrophages and lymphocytes), and edema in the dependent lung [15]. In an experimental study, these changes were observed within 90 min from the initiation of mOLV [21]. As these immune cells become activated, an inflammatory cascade is induced as a part of the biotrauma of mOLV. In addition, hyperperfusion of the dependent lung develops during mOLV. When hyperperfusion is combined with hyperinflation, alveolar damage occurs, causing interstitial edema and microhemorrhages. Intra-alveolar and inflammatory cells are part of the biotrauma caused by mOLV; other components include cytokine release and inflammatory response reactions [15,16]. As with the other disadvantages of mOLV (volutrauma and atelectrauma), biotrauma can be reduced by performing NITS.

2.4. Physiological changes in mOLV

Ventilation and perfusion are the most important functions of the lung; due to their close correlation, they are measured together as the ventilation/perfusion (V/Q) ratio. Similar to the pathological changes noted above, the physiological changes during mOLV are caused by damage to the alveoli (volutrauma/barotrauma). A V/Q mismatch can occur during the different periods of the mOLV or sOLV procedure, and many factors influence it (patient position, exploration of the thoracic cavity, and surgical manipulation of the operated lung). The special characteristics of ventilation during mOLV are detailed above. The pulmonary artery, pulmonary vein, and alveolar pressures have a central role in perfusion. In cases of increased lung volume, the alveolar capillaries are compressed, and the pulmonary vascular resistance increases. Hypoxic pulmonary vasoconstriction (HPV) is the oxygen-sensing mechanism of the lung that reduces perfusion at the hypoxic part of the lung and drives it to a better-ventilated area. The result of these factors is a V/Q mismatch. In an experimental study, mOLV was shown to cause a V/Q mismatch, with hyperperfusion and alveolar damage in the dependent lung [21]. The beneficial effect of spontaneous ventilation on the V/Q ratio can be demonstrated in patients with acute respiratory distress syndrome. Spontaneous ventilation increases the ventilation and perfusion rates and improves the heart function and oxygenation [22]. The same effect can also be observed in sOLV cases. The complications of mOLV in the dependent lung can disappear or diminish in NITS with sOLV, but other emerging disadvantages such as hypoxia and hypercapnia must be considered. Hypercapnia is one of the most frequent indications for converting to mOLV in cases of NITS, but the occurrence of permissive hypercapnia is a well-detailed and accepted side effect in NITS. It can be managed by re-expansion of the lung with intermittent non-invasive ventilation before the final indication for conversion occurs [23].

2.5. Cardiac and hemodynamic effects of mOLV

As discussed above, the change in pressure in the thorax has a central role in the regulation of the lung and heart functions. 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 [18]. 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. For this reason, there are more frequent but transient cardiac instabilities only in this part of the NITS procedure, when the chest has just been opened. 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. However, this kind of temporary instability can occur differently between epidural and paravertebral/intercostal + vagus nerve block methods used in NITS procedures [24-28]. The positive hemodynamic effect of the epidural NITS method has previously been emphasized [25].

2.6. 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 improved left ventricular function in coronary artery disease, decreased cardiac morbidity and mortality, fewer postoperative pulmonary complications, and adequate pain management [25, 27]. However, there are limitations to the application of TEA, from spinal cord injury to epidural bleeding/hematoma and infection [29]. 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 [28, 30]. Another positive pathophysiological effect of TEA is its sympatholytic effect, which can also reduce surgical stress [25] and reduce troponin T and C-reactive protein (CRP) levels [31, 32]. Moreover, in cases of mOLV during esophagectomy, TEA significantly reduced the levels of

pro-inflammatory cytokines (interleukin IL-6 and IL-8) [33]. Theoretically, this effect could be more effective in sOLV. Intercostal nerve block can also significantly reduce the stress response, e.g. reducing the levels of IL-6 and TNFα (tumor necrosis factor α) [34]. In contrast, a meta-analysis showed no significant difference in postoperative inflammatory response (IL-6 and CRP levels) between the different types of anesthesia [35].

2.7. 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), also called alarmins. DAMPS released from damaged tissue are recognized by TLRs (Toll-like receptors). These receptors are found on the surface of macrophages and dendritic cells. Alarmins can also bind to intracellular NOD receptors (nucleotide-binding oligomerization domain-containing protein). Ligation of the DAMPS to TLR or NOD receptor activates the production of the proinflammatory cytokines (IL-1, IL-6, TNF-α) [1]. 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), and the spread of cancer. 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). The accepted view was that: the initial phase of SIRS is followed by CARS, but nowadays the view of the paralell development of SIRS and CARS dominates [1, 8].

After surgery, an increased number of leukocytes and a reduced number of lymphocytes indicate deep immunosuppression; the lower number of lymphocytes is caused by postsurgical apoptosis [1]. Native CD4+ Th0 are bipotential, these cells are the precursors of Th1 and Th2 cells. The antigen-presenting cells (APC) such as monocytes/macrophages and dendritic cells present antigens to CD4+Th0. Depending on the antigen, Th0 cells differentiate with the help of cytokines (IL-12, IL-4) into Th1 or Th2 cells. Th1 cells produce pro-inflammatory cytokines, such as interleukin-2 (IL2), interferon-gamma (IFN-γ), and tumor necrosis factor-beta (TNF-β), these cytokines are important for killing intracellular pathogens and tumor cells. Th2 cells produce anti-inflammatory cytokines, such as IL-4, IL-5, IL-10, and IL-13, these cytokines play an important role in antibody production and in defense against extracellular parasites. The

antigen-presenting cells are components of the innate immune system, while T cells are components of the acquired (adaptive) immune system [8, 36]. (Figure 3.)

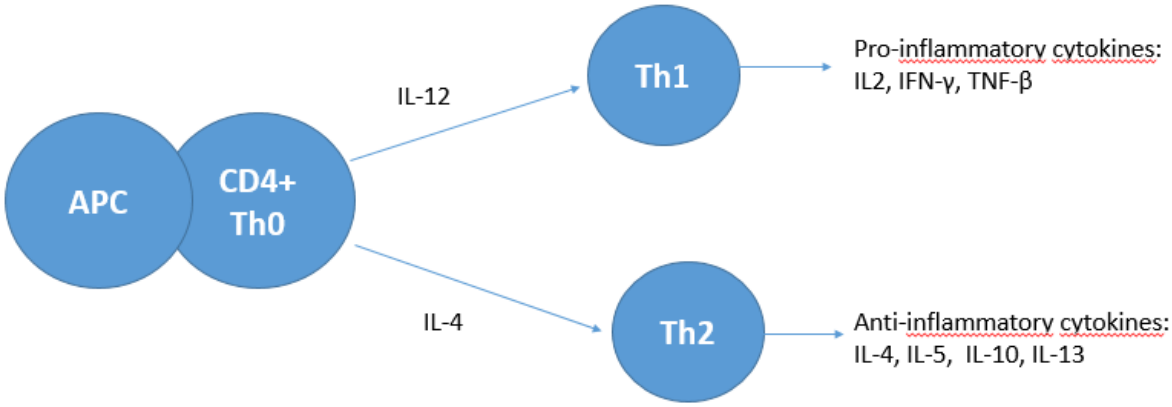

Figure 3. Development of Th1 and Th2 cells. Abbreviations: APC antigen-presenting cell, CD cluster of differentiation, Th T helper cell, IL interleukin, IFN-γ interferon-gamma, TNF-β tumor necrosis factor-beta.

A significantly reduced Th1/Th2 ratio observed on the second postoperative day until the fourteenth day after surgery. The suppression of Th1 response and the intensification of the T2 response can be an important factor in postoperative complications (wound infections, pneumonia, septicaemia, tumor growth) [1]. (Figure 4.)

These changes in the levels of leukocytes, natural killer cells [37], lymphocytes, and cytokines have been investigated by several studies [38, 39] that showed that these changes were lower after NITS than in relaxed-surgery cases [40]. The reduced inflammatory and immune changes after NITS suggest that immunosuppression is reduced after NITS compared to relaxed-surgery cases [39, 41].

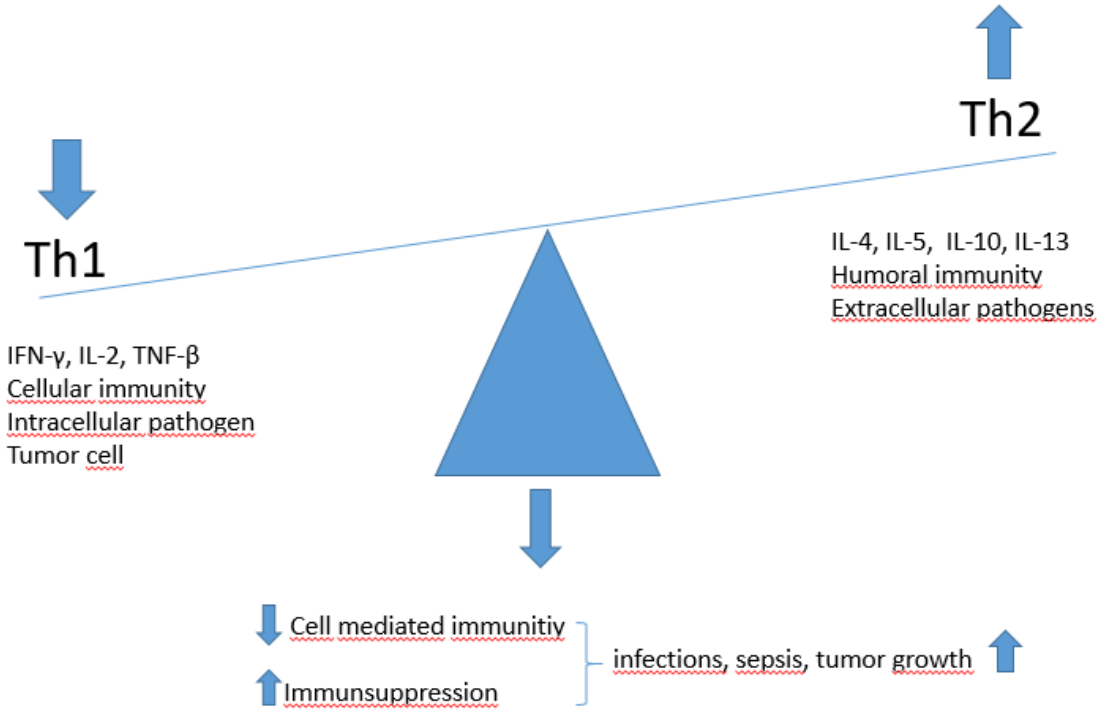

Figure 4. Th1/Th2 imbalance after surgey. Abbreviations**:** Th T helper cell, IFNγ interferon gamma, IL interleukin, TNF-β tumor necrosis factor-beta.

2.8 Postoperative neuroinflammation and cognitive impairment

Postoperative SIRS also affects the central nervous system. The proinflammatory cytokines (e.g. TNF-α, IL-6) increase the permeability of the blood-brain barrier (BBB) and cause neuroinflammation. As a result of neuroinflammation neuronal apoptosis increases, hippocampal neurogenesis and the number of synaptic connection decreases. Together, these factors increase the risk of postoperative cognitive dysfunctions (POCD) and the risk of the Alzheimer's disease [42] (Figure 5). According to studies by Vanderweyde et al. 1/3 of patients aged 65 and over suffer from POCD, with 70% of these patients developing dementia 3 to 5 years later [43].

Nowadays, according to WHO (World Health Organization) data about 55 million people suffer from dementia, and approximately 10 million new cases are diagnosed each year. Due to the aging of the population, an increase in the incidence of dementia is expected (in 2030- 78 million, in 2050 - 139 million patients)

Alzheimer's disease accounts for 60-70% of all dementias. The seventh leading cause of death is dementia [44]. 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.

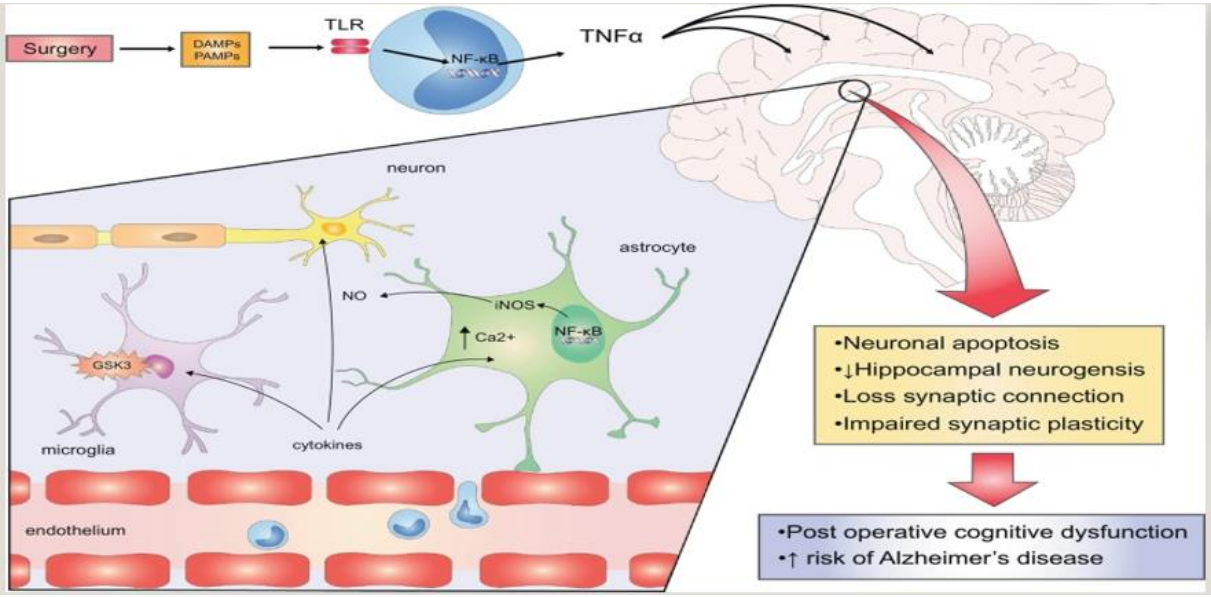

Figure 5. Mechanism of postoperative neuroinflammation [42]. Proinflammatory citokines, such as TNFα are produced as a result of PAMPs and DAMPs. Blood-brain barrier (BBB) is damaged, the number of lymphocytes increases in the central nervous system and astrocytes and microglia are activated. Nitric oxide (NO) is released from activated astrocytes and microglia. Intracellular Ca^{2+} also increases under the influence of cytokines. Neuroinflammation cause GSK-3 dysfunction, as a result of these dysfunction microglial migration and activation increases. Abbreviations: BBB blood-brain barrier, CA^{2+} calcium, DAMP danger/damage associated molecular pattern, GSK-3 glycogen synthase kinase-3, iNOS inducible nitric oxide synthase, NO nitric oxide, PAMP pathogen associated molecular pattern, TLR toll like receptor.

2.9. Effects of NITS on cancer

Only few studies have mentioned the short- and long-term effects of NITS on cancer behavior. One of the first findings on this topic was better survival after NITS compared to relaxed-surgery in cases of malignant pleural effusion [40]. In a clinical study, better compliance with

adjuvant chemotherapy with less toxicity after NITS lung lobectomy was verified, and 92% of patients who underwent NITS were able to receive the planned chemotherapy protocol, compared to 72% of relaxed-surgery cases [45]. In another study, the overall survival and disease-free survival after lung cancer surgery were significantly better after sOLV than after mOLV, with type of anesthesia being an independent factor for both overall and disease-free survival in patients who had spontaneous ventilation [46]. However, another study demonstrated no significant difference in disease recurrence and survival between the sOLV and mOLV methods [47]. Meanwhile, a study on awake breast cancer surgery suggested that both extrathoracic cancer cases and lung cancer sOLV cases had shorter operative time and length of hospital stay [48, 49]. Additionally, a case series presented about successful esophagectomy wherein the thoracic part was performed under spontaneous ventilation [50]. The role of cytokines in cancer regulation is well detailed in terms of molecular background [51, 52], especially IL-6, which has a central role and has been widely investigated in different clinical studies [10, 12, 38, 41]. IL-6 plays an important role in cancer cell communication. IL-6 promotes tumor progression and the dissemination of metastases. It also has a significant systemic effect: failure of metabolism, mental problems of cancer patients [53]. Th1 immunity and Th2 immunity approach a balance. But, the the presence of tumor cells and the surgery disrupts this balance. Th2 immunity increased and decreased Th1 immunity, and this leads to tumor progression. If Th1 immunity becomes predominant, this stimulation of immunity can lead to tumor regression [36] (Figure 6).

Cytokines can activate carcinogenesis and tumor growth and can protect cancer cells from therapy-induced gene damage and apoptosis [54]. The levels of these released cytokines have been confirmed to be lower in sOLV cases than in mOLV cases, thus causing less damage on the anticancer activity of the body and promising better long-term results after NITS.

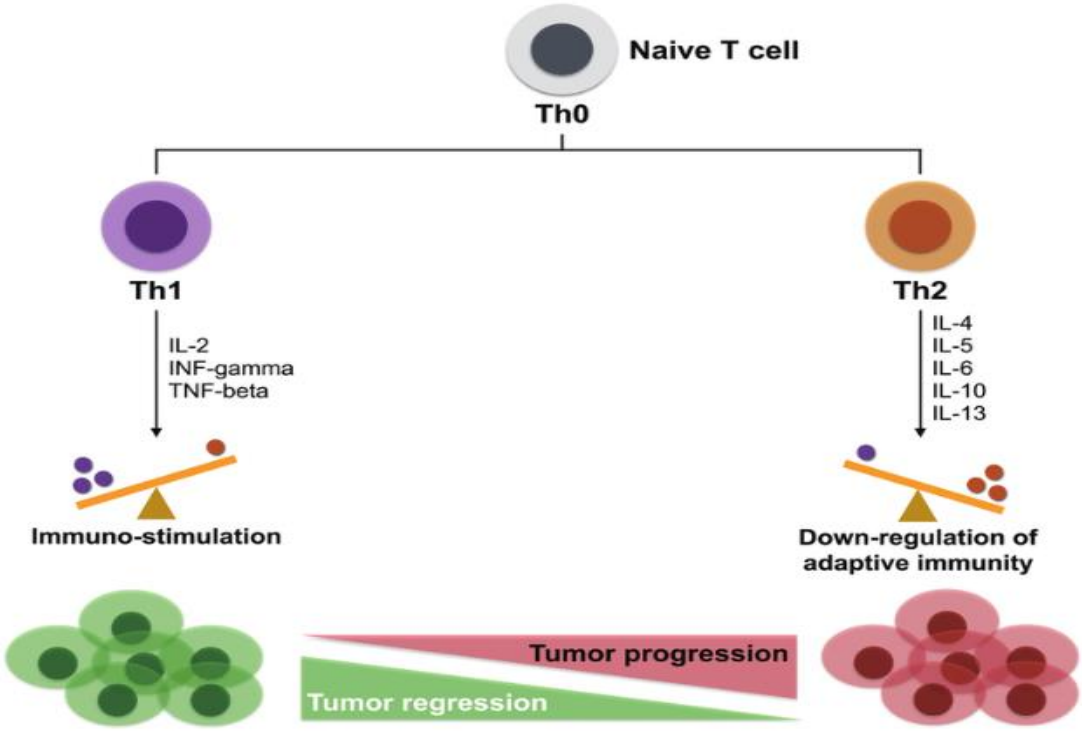

Figure 6. Effects of Th1/Th2 balance on tumor progression [36].

Abbreviations**:** IFN-gamma interferon gamma, IL interleukin, Th T helper cell, TNF-beta tumor necrosis factor-beta.

2.10. 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 [55,56], 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 (Figure 7.). 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.

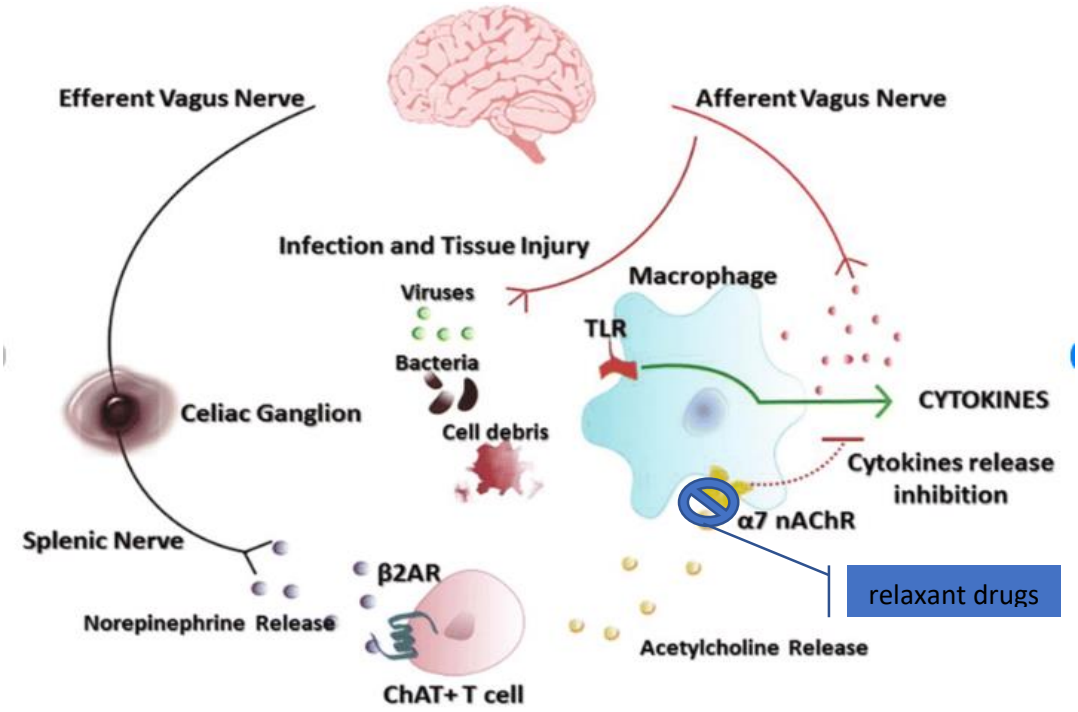

Figure 7. The cholinergic anti-inflammatory pathway [56]. *„The afferent arm of the vagus nerve activated by peripheral inflammatory stimuli (i.e., infection and tissue injury) signals the nucleus tractus solitarius that, by stimulating the efferent vagus nerve, causes norepinephrine release from the vagal splenic terminations. This in turn stimulates ACh secretion by the ChAT*$^{+}$ *T cells in the spleen that by binding α7nAChR expressed by macrophages induces anti-inflammatory response."*Abbreviations: ACh acetylcholine, ChAT+ Tcell choline acetyltransferase-expressing T cells, α7nAChR α7 nicotinic acetylcholine receptor.

2.11. The summary advantages of NITS

Performing NITS with spontaneous ventilation can prevent or reduce volutrauma 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 can moderate the immunosuppression caused by mechanical ventilation. 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 reduced pro-inflammatory response and cytokine release can affect the central nervous

system and the spread of cancer. 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. The advantages of NITS are discussed in the context of the negative effects of mOLV, which can be potentially reduced by performing NITS. However, some direct clinical and pathophysiological arguments (reduced inflammatory response, limited change in the number of leukocytes, and fewer postoperative morbidities) can support our theory that the NITS procedure is more physiological than mOLV. Survival in malignant pleural effusion [40] and lung cancer resection [46] improved more with NITS than with relaxed surgery, whereas compliance with adjuvant chemotherapy was better in patients who underwent NITS than in mOLV cases [45]. It must be noted that the locoregional anesthesia in NITS is associated with a reduced cytokine release, contributing to a more physiological postoperative immune function. However, there are only a few centers where the NITS procedure is routinely performed, and we could not find many prospective randomized studies about the direct advantages of sOLV. Furthermore, despite the effects of reduced surgical stress, the potential advantages of NITS are still not widely applied in daily clinical practice in Europe [57]. Both immunological and physiological aspects of the topic could be examined by animal and laboratory studies. Third is the possible improvement in the “NITS theory” by demonstration of its direct clinical impact on 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. However, according to the NITS theory, the immunosuppression is less after sOLV than after mOLV, suggesting that after NITS the postoperative immunotherapy should be more effective.

3. SPONTANEOUS VENTILATION COMBINED WITH INTUBATION

3.1. Background

Mechanical one-lung ventilation (mOLV) has several disadvantages; thus, in the last few decades, thoracic surgeons have pursued new techniques for even great interventions of the thorax in local anesthesia and spontaneous ventilation. In the early 2000s, a group at Tor Vergata University published their first results regarding awake non-intubated patients [58]. In 2014, Gonzales-Rivas first performed non-intubated uniportal video-assisted lobectomy [13]. In the last few years, this technique has gained approval in several medical facilities. Studies have shown that this method yields better clinical and immunological results [40, 59]. Despite the reported advantages, the technique still causes disputes between anesthesiologists because of potential airway loss and the complicated manner of conversion to traditional one-lung mechanical ventilation [14, 60]. These concerns may prevent the widespread application of this method. Our department reported excellent clinical and oncological results among our non-intubated thoracic surgery (NITS) spontaneously ventilated patients [45, 61]. Therefore, 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.

3.2.Patients and methods

3.2.1. Spontaneous ventilation combined with double-lumen tube intubation

Between March 11, 2020 and March 26, 2021, 68 SVI thoracic surgeries were performed in our department (Table 1). We used the VATS method in 89.7% (n=61) of the cases, and 62.3% (n=38) of the SVI VATS cases were lobectomies. In this study, 38 SVI VATS lobectomies were analyzed. The SVI VATS method was previously published [62]. 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 (left side in the aortopulmonary window, right side in the upper mediastinum). 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. The pCO_2 and oxygen saturation could be kept within or close to the normal range with a higher FIO2 (40–100%) and a positive end expiratory pressure (PEEP; 3–5 H_2Ocm) in the dependent lung (if required), which was easily measured through the double-lumen tube. If necessary, pressure support ventilation (PSV) without relaxation or recruitment maneuvers can be performed to maintain normal oxygen and CO_2 levels. Anaesthesiologic indications for conversion to relaxation and mOLV are hypoxemia and hypercapnia. For SVI VATS lobectomies, we used the same oncological inclusion criteria as in the NITS cases [63]. We considered a body mass index (BMI) of more than 30 as the only exclusion criterion for SVI cases, and the other exclusion criteria of the NITS were not contraindications for SVI procedures (Table 2).

SVI (n=68)	**Intended open SVI (n=7)**	**VATS SVI (n=61)**
Lobectomy	4	38
Segmentectomy	1	7
Atypical resection	0	11
Thymectomy	0	5
Lymph node block dissection	2	0

Table 1. SVI surgeries.

Abbreviations**:** SVI spontaneous ventilation with intubation, VATS video-assisted thoracic surgery.

NITS	SVI
BMI≥ 30 kg/m2	BMI≥ 30 kg/m2
Hemodynamically unstable patients	
Persistent cough or high airway secretion	
Anticipated difficult airway	
INR> 1.5	
Sleep apnea syndrome	
Raised intracranial pressure	
Patients unable to cooperate	
Elevated risk of regurgitation	
Procedures requiring lung isolation to protect the contralateral lung	

Table 2. Exclusion criterias from NITS and SVI.

Abbreviations**:** BMI body mass index, INR international normalised ratio, NITS non-intubated thoracic surgery, SVI spontaneous ventilation with intubation.

3.2.2. Non-intubated thoracic surgery

The first 38 NITS VATS lobectomies were performed in our department between February 2, 2017 and September 14, 2017 and compared to the 38 SVI VATS lobectomies mentioned above. The chosen NITS VATS lobectomy results were already presented at the 2018 European Society of Thoracic Surgery annual meeting in Ljubjana. The NITS patient selection criteria were based on the currently recommended selections [11]. 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) [63]. The surgical procedure is similar to that of SVI, but in NITS cases at the beginning of the surgery, the patient is not relaxed and intubated; therefore, manipulation of the vagus and intercostal blockade must be very careful.

3.3. Anesthesiology of SVI

In addition to general standard monitoring, the depth of anesthesia was controlled by the BIS (Medtronic Vista), and invasive blood pressure measurements were performed. Fentanyl and midazolam were administered prior to surgery. Anesthesia was induced and maintained with propofol to maintain a BIS of 40–60 [59]. After the adequate depth of anesthesia was achieved, a laryngeal mask was inserted to maintain a clear airway. The oxygen and air mixture was supplemented via a T-piece, and FiO2 was titrated to keep the SpO2 at above 92%. In both groups, phenylephrine (50–100 mcg) in divided doses was administered if systolic blood pressure decreased to less than 100 mmHg, decreased by more than 25%, or the mean arterial pressure was less than 60 mmHg. In this study, there were two diferent periods of time with NITS and later SVI procedures. There was no period where mixing of the two types of surgeries took place. The preoperative check-up was the same as that in the case of standard lobectomy in both groups. The comorbidities of the selected patients were characterized using the Carlson Comorbidity Index. The lobectomies were performed using the uniportal VATS technique in both groups. In all cases, digital suction was applied with a negative pressure of 15 W. The drain was removed when there was no air leak and the daily fuid volume was less than 400 mL. During the surgery, pulse, blood pressure, oxygen saturation, pCO2, and ventilation rate were analyzed in both groups. Patient data and the lobectomy types are presented in Tables 3 and 4.

	NITS VATS (n=38)	**SVI VATS (n=38)**	***p* value**
Female/male	22/16	18/20	0.253
Age	64.9	65.4	0.842
BMI	25.0	26.7	0.060
CCI	5.6	5.0	0.157
FEV1 (%)	90.4	87.1	0.470

Table 3. Data of the NITS and SVI lobectomy patients

Abbreviations: BMI body mass index, CCI Carlson Comorbidity Index, FEV1 forced expiratory volume in 1 s, NITS non-intubated thoracic surgery, SVI spontaneous ventilation with intubation, VATS video-assisted thoracic surgery.

	NITS VATS (n=38)	SVI VATS (n=38)	*p* value
Right upper lobe	14	6	0.038
Right middle lobe	5	7	0.528
Right lower lobe	6	14	0.038
Left upper lobe	7	7	1.00
Left lower lobe	6	4	0.5

Table 4. Data of the NITS and SVI VATS lobectomies

Abbreviations: NITS non-intubated thoracic surgery, SVI spontaneous ventilation with intubation, VATS video-assisted thoracic surgery.

3.4. Results

The intraoperative anesthesiology parameters of the NITS and SVI groups are presented in Table 5. 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).

	Analyzed cases in NITS group	**NITS**	**Analyzed cases in SVI group**	**SVI**	***p* value**
Pulse minimum (/min)	32	60.3 (45-80)	36	63.6 (40-90)	0.217
Pulse maximum (/min)	32	84.5(65-105)	36	82.9 (58-130)	0.582
Lowest systolic BP (mmHg)	32	83.1(65-115)	34	132.3 (90-180)	**0.0001**
Lowest diastolic BP (mmHg)	32	47.8(35-70)	34	73.4 (45-120)	**0.0001**
Circulation support case	38	21 (55.2%)	38	15 (39.5%)	0.171
Lowest O2 saturation (%)	32	90.3 (44-99)	36	94.9 (96-100)	**0.026**
Highest O2 saturation (%)	32	98 (86-100)	36	99.4 (96-100)	0.065
pCO2 minimum (kPa)	31	39.6(28.5- 53.7)	32	39.1 (28-49)	0.692
pCO2 maximum (kPa)	31	62.5 (47-106)	33	54.8 (37.7-90.9)	**0.009**
Ventilation rate minimum (/min)	9	12.7 (9-18)	36	11.9 (6-18)	0.448
Ventilation rate maximum (/min)	9	19.2 (16-24)	35	18.2 (12-30)	0.424

Table 5. Data of the cardio-pulmonary functions.

Abbreviations: BP blood pressure, NITS non-intubated thoracic surgery, O_2 oxygen, pCO_2 partial pressure of carbon dioxide, SVI spontaneous ventilation with intubation

The perioperative results are shown in Table 6. We did not find significant differences in operation time, duration of drainage, or morbidity, but the surgical and drainage times were slightly shorter in the SVI group. The morbidity rate was 21% in the NITS group and 13.1% in the SVI group (p=0.364). Postoperative complications in the NITS group were as follows: prolonged air leak (n=6), readministration due to pneumothorax with the need for chest drainage (n=1), and bleeding that required reoperation (n=1). In the SVI group, the following complications occurred: prolonged air leak (n=4) and new-onset atrial fibrillation (n=1).

	NITS VATS (n=38)	**SVI VATS (n=38)**	***p* value**
Surgical time (min)	98.7 (55-180)	88.1 (55-120)	0.067
Drainage time (day)	3.5 (1-12)	2.7 (1-10)	0.194
Morbidity	8/38 (21%)	5/38 (13.1%)	0.364
PAL	6/38 (15.7%)	4/38 (10.5%)	0.5
mOLV conversion rate	2/38 (5.2%)	5/38 (13.1%)	0.237
Failure of SVI method	4/38 (10.5%)	5/38 (13.1)	0.724

Table 6. Perioperative results of the NITS and SVI lobectomies.

Abbreviations: mOLV mechanical one-lung ventilation, NITS non-intubated thoracic surgery, PAL prolonged air leak, SVI spontaneous ventilation with intubation, VATS video-assisted thoracic surgery.

The histological results are shown in Table 7. 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).

	NITS VATS (n=33)	SVI VATS (n=26)
Adenocarcinoma	28	19
Squamosus cell carcinoma	2	4
Small cell lung cancer	0	1
Large cell neuroendocrine carcinoma	0	1
Typical carcinoid	2	1
Carcinosarcoma	1	0
IA	18	11
IB	9	2
IIA	0	2
IIB	1	5
IIIA	5	4
IIIB	0	1
IV	0	1

Table 7. Histological results of the primary lung cancer cases.

Abbreviations**:** NITS non-intubated thoracic surgery, SVI spontaneous ventilation with intubation, VATS video-assisted thoracic surgery.

3.6 The summary advantages of SVI

The main goal of the development of the SVI procedure was to ensure airway safety during spontaneous ventilation thoracic surgery. In our previously published data using the SVI technique, we reduced the duration of mechanical one-lung ventilation by 76.6%, thereby lowering the changes in pulmonary physiology [62]; However, insufficient information was available to determine whether SVI has other advantages over reduction of the mechanical one-lung ventilation period. The main criticism against SVI is that sufficient spontaneous ventilation through one side of the double-lumen tube is impossible. In this study, there were ten cases (26.3%) of spontaneous ventilation via the double-lumen tube in which a PEEP of 3-5 cmH_2O and FIO_2 of 40–100% were insufficient to maintain the acceptable gas exchange parameters, but it was successful in 73.7% of cases. In addition, 13.1% of the spontaneous ventilation supported with PSV resulted in a normal gas exchange rate. Thus, acceptable gas exchange without the need for conversion to relaxation was provided in 86.8% of the SVI cases and 94.8% of the NITS cases. Although the oxygen saturation and the pCO_2 levels were in the normal

range in both groups, intergroup differences were significant in the lowest oxygen saturation and highest pCO_2 levels. The mean lowest oxygen saturation was significantly higher in the SVI group than in the NITS group (94.9% vs. 90.3%) (p=0.026), while the highest mean pCO_2 level was significantly lower in the SVI group than in the NITS group (54.8 *vs* 62.5 *vs* kPa) (p=0.009). 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.

Regarding the SVI technique, the question arises as to how patients do not cough during SVI surgery after ipsilateral vagus blockade, when the contralateral side main stem bronchus is stimulated by the double-lumen tube. We observed that, during the surgery, there was no cough if we did not directly move the ipsilateral main stem bronchus; However, if this kind of movement occurred, which moved the contralateral bronchus as well, the patient coughed. We suggest that this movement causes a change in the location of the balloon of the tube in the contralateral bronchus, and this movement of the tube generates the cough reflex. However, if we moved the lobes (for example, the right upper lobe) during resection and stapled the lobal bronchus, there was no evident cough. Our theory regarding this kind of cough is supported by the literature. The cough receptors (terminals of Aδ-fiber with circumferential arborization in the main airway) are localized almost exclusively to the extrapulmonary bronchi and trachea [64]. They are sensitive to punctate mechanical stimulation (and rapid changes in luminal pH) and can be activated by a vertical dynamic force. As the force becomes static, activation of the receptor ceases [65, 66]. With this extrapulmonary Aδ nodose mechanosensitive vagal afferent nerve pathway, cough can be produced in anesthetized animals as well [65]. Based on the above discussion, in our SVI procedure, the cough refex cannot be prevented completely, but it can be reduced to a very low threshold with ipsilateral vagus blockade and low BIS caused by sedation. If dynamic mechanical stimulation of the contralateral main stem bronchus occurs, a cough will also occur; however, if the location of the double tube cuf in the contralateral main stem bronchus remains static, the cough refex is not activated.

Regarding the ventilation, there is another question about the advantage of SVI with use of PEEP and PSV over relaxed cases. Mechanical ventilation, specifically mechanical one-lung ventilation, may cause overdistension of the alveoli due to high peak pressure and pressure differences and presence of an opening/closing phenomenon in the alveolar system due to ineffective levels of PEEP. The main advantage of SVI ventilation is the regulation of inspiration–expiration. In SVI, the same PEEP and PSV are used if needed, as in the relaxed cases, but the volume and time period of ventilation are controlled by the patient, rather than ventilator parameters. In SVI cases, if the lung stretching receptors detect that the lung

parenchyma is overdistended, the patient will stop inspiration with the Hering–Breuer reflex, preventing barotrauma in the lung parenchyma. The ventilator cannot detect this threshold, and it would incorrectly provide more volume, causing overdistension, or less volume, resulting in lower airway pressure leading to atelectasis.

Regarding the anesthesia, further advantage of the SVI over the mOLV, in addition to enabling ventilation regulation by the patient, is the reduced period of relaxation (75% in SVI compared to the traditional mOLV). If the theory of volutrauma of the alveoli caused by mOLV can be accepted as a real pathophysiological phenomenon, the time required for alveolar injury in SVI cases is reduced to 75% and the SVI procedure can attenuate the proinflammatory response. An animal study stated that the cytokine release in mOLV begins within 90 min after the relaxation [67]. In that case, the SVI can reduce the changes in the cytokine release, as it has a mean relaxation time of 25.5 min, as mentioned in our previous study [62]. The pressures applied in SVI (3-5 PEEP and PSV) correspond to the requirement of the protective ventilation during mOLV [68]. Based on the pressures in SVI, it can at least be stated that the SVI is not more harmful than the traditional mOLV. The quantity of the relaxant drug used is a signifcant difference between the SVI and mOLV. A much smaller amount of the relaxant drug is used in SVI than in mOLV. This is very important because the relaxant drug can cause proinflammatory cytokine release directly from the macrophages. It has been proven that acetylcholine (ACh) reduces the release of the proinflammatory cytokines from macrophages in a culture on binding to their α7ACh receptors (detailed above cholinergic anti-infammatory pathway) (Figure 7.) [55]. The relaxant drugs use the same receptor in the neuromuscular junction; if they block the function of the ACh on macrophages, cytokine release can be induced, with its well-known side effects.

The better oxygenation and carbon dioxide exchange of the SVI provides the basis for another very important advantage of the SVI: better cardiopulmonary stability during spontaneous ventilation thoracic surgery. In the SVI cases, the lowest systolic and diastolic blood pressure values during surgery before its medical correction were significantly higher than those in the NITS cases (systolic: 132.3 vs. 83.1 mmHg, p=0.001; diastolic: 73.4 vs. 47.8 mmHg, p=0.0001), which means that cardiac function is more stable during the SVI procedure than during the NITS procedure.

In both procedures, the patients are spontaneously ventilated, so the cardiac alteration caused by mediastinal movement can be excluded. Another reason could be the change in cardiac innervation, but it can also be excluded because the same vagus and paravertebral blockade with the same amount of bupivacaine was applied in both methods. In our opinion, the basis of

this stability in SVI cases is the better oxygen saturation level, caused by 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 (HPV), thus it decreases less the blood pressure [69].

Regarding cardiac function during thoracic surgery in SVI, NITS, and relaxed cases, the intraoperative period can be subdivided into three sessions.

The first session lasts until the chest is opened. In this session, the only major change in the hemodynamic condition is a decrease in systemic vascular resistance causing lower afterload. This occurs due to administration of drugs for anesthesia induction, regardless of the type of surgery; hence, this period is the same in SVI, NITS, and relaxed cases.

The second session begins after the chest has been opened. If the chest is opened, signifcant hemodynamic changes occur due to a loss of negative intrapleural pressure and lung collapse causing HPV, resulting in an increase in pulmonary vascular resistance (PVR) and decrease in venous return. If the patient is ventilated in relaxed cases or supported by PEEP and PSV in SVI, the positive pressure and applied PEEP can diminish the effect of these factors. In NITS, transient hemodynamic instability may occur depending on the patient's cardiac condition, intraoperative systemic and pulmonary resistance, and pressure changes during chest opening and lung collapse. This may require the use of vasoconstrictor drugs to stabilize cardiac output. Medical support for cardiac function can thus become more intensive in NITS cases than in intubated patients.

The third session is the stable period after hemodynamic balance is achieved; it is a safe and relatively uneventful period until conclusion of the operation in SVI, NITS, and relaxed cases.

According to our clinical observations, fewer vasoconstrictor drugs are needed during SVI procedures as compared with NITS. We used the same drug dosage for pain control and sedation, and there was no difference between the targeted anesthetic depth. The only differences was the initial mechanical ventilation in the early SVI period until the muscle relaxant was eliminated, and the administration of PEEP and PSV if needed. Based on these data, we believe that PSV and PEEP, if required, cause physiological changes that can stabilize cardiac function during spontaneous ventilation [70, 71].

The perioperative results of the SVI group were similar to those of the NITS group. This technique could also be used in oncology patients with nearly the same outcomes. The two groups had no signifcant differences in surgical time or postoperative period. Our SVI lobectomy results were comparable with the NITS lobectomy results reported by other groups. Hung et al. reported a drainage time of 2 days [26], while Jun Liu et al. stated 3.2 days [59]

compared to the 3.5 and 2.7 days after NITS and SVI lobectomies in our patients The postopeartive morbidity rate was around 9-12% in the previously mentioned papers compared to our 13.1% result. We found the advantage of SVI in prolonged air leak (PAL) compared to our NITS cases (10.5% vs 15.7%), and PAL in Hungs (11.9%) [26] or AlGhamdis NITS cases (16.7%) [72]. However, the mean surgical time was longer in these studies, 124–177 min, than in our study (98.7 and 88.1 min in the NITS and SVI groups, respectively) [26, 59, 72]. We compared perioperative result of SVI to those of VATS lobectomies with mOLV (Table 8). Analysis of our two studies showed, that drainage time after SVI decreased almost by 50% compared to mOLV cases (2.7 vs. 5.1 days), with operative time and postoperative morbidity showing no significant difference [73]. Comparing results of our uniportal VATS SVI lobectomies to the same data of other uniportal and multiportal VATS mOLV studies, shorter opeartive time after SVI was found, however in corellation with literature data, drainage time and postoperative morbidity had similar outcomes [72, 74]. According to our results, SVI has favourable, and at least similar perioperative results compared to mOLV.

	SVI VATS uniportal lobectomy (n=38) our study	mOLV VATS uniportal lobectomy (n=81) Furák [71]	mOLV VATS uniportal lobectomy (n=92) Gonzales-Rivas [72]	mOLV VATS multiportal lobectomy (n=30) AlGhamdi [70]
Surgical time (min)	88.1 (55-120)	96.6 (44-188)	154.1 (60-310)	146± 47.4
Drainage time (days)	2.7 (1-10)	5.1 (1-25)	2 (1-16)	5.4 ± 5.4
Morbidity (%)	13.1	12.3	15.2	20

Table 8. Perioperative results of the SVI and mOLV VATS lobectomies.

Abbreviations: mOLV mechanical one lung ventilation, SVI spontaneous ventilation with intubation, VATS video-assisted thoracic surgery.

4. THE COVID-19 PANDEMIC AND THE THYMUS

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.

4.1 Background

As the center of T cell differentiation, the thymus may play an important role in the immune response to coronavirus infection. 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. 90% of the T cells are destroyed and only the remaining 10% enter the circulation [75]. 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 [75].

The maturation of natural killer (NK) cells also occurs in the thymus, which cells are the link between the innate and acquired immune systems. They can produce pro- and anti-inflammatory cytokines, but can also kill target cells, and their surface is CD56+, CD3- and CD25+ [75]. 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 [75].

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 [76-78].

The SARS-CoV-2 virus reduces the antigen presentation to cytotoxic T cells, thereby reducing the antiviral effect of T cells [79]. 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 [79-83]. 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 [79], 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 [84].

Computed tomography (CT) of the chest and reverse transcription-polymerase chain reaction (RT-PCR) play the important role in the diagnosis and management of COVID-19 disease. These CT scans provide an opportunity not only for the imaging of the lungs, but also of the thymus. The most common radiological changes in the thymus of COVID-19 patients were the following: solid soft tissue replacement of the thymic gland, mixed soft tissue and fatty replacement thymic gland, nodular infiltration of the thymic gland (Figure 8) [85].

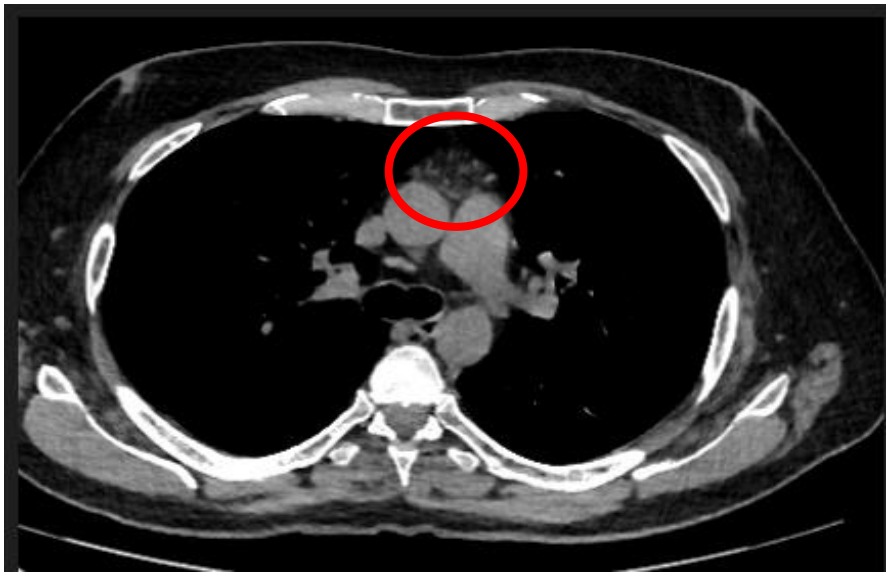

Figure 8. Thymus CT in COVID-19. Axial mediastinal window CT showed nodular pattern (infiltration) of the thymus. Abbreviations: COVID-19 coronavirus disease 19, CT computed tomography.

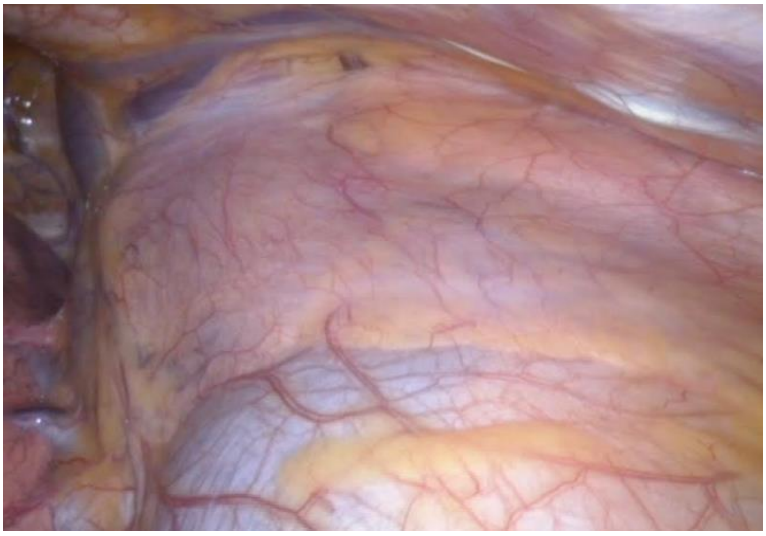

Figure 9. Intraoperative view of thymus hyperplasia.

4.2 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 (Table 9)

	PC	NC	VIC
Female	12	11	4
Male	10	9	9
Age	29.2	45.7	49.7
VATS-thymectomy	17	20	10
Partial sternotomy	5	0	3

Table 9. Data of the patients and surgical methods.

Abbreviations: PC pre-COVID-19 group, NC no-COVID-19 group, VIC vaccinated or infected COVID-19 group, VATS video-assisted thoracic surgery.

4.3 Pathological methods

Haematoxylin-eosin stained slides were re-evaluated and tissue microarray (TMA) blocks were created. Two core samples were taken from each case to represent sufficient amount of thymus tissue. CD4, CD8, CD25 and FOXP3 immunohistochemical reactions were performed. Table 10. displays the technical details of the immunohistochemical reactions. The intensity of staining was evaluated by 2 independent pathologist on a four tiered scale (0-3+). Discrepancies were discussed at a two-headed microscope (Olympus BX43, Tokyo, Japan). Kruskal-Wallis tests were performed to identify significant differences. SPSS software was utilized (IBM, SSPS 22.0, Armonk, NY, USA). Significance level was $p < 0.05$.

Antibody	Clone	Manufacturer	Dilution	Antigen retrieval
CD4	SP35	Cell Marque	1:100	pH9; 20 min
CD8	C8/144B	DAKO	1:100	pH9; 20 min
FOXP3	259D	BioLegend	1:100	pH10; 20 min
CD25	4C9	LabVision	1:50	pH6; 60 min

Table 10. Details of immunohistochemical reactions applied.

Abbreviations: CD cluster of differentiation, IgG immunoglobulin G, FOXP3 forkhead box P3.

4.4. Results

4.4.1. 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 (Diagram 1).

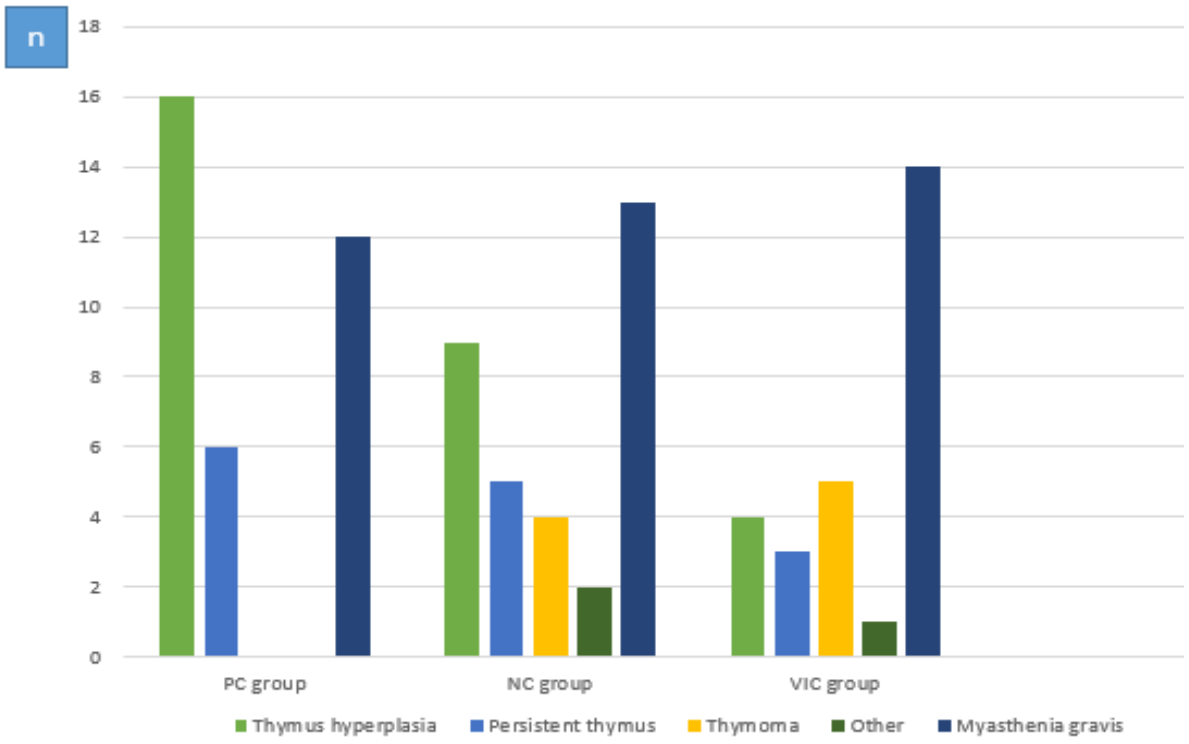

Diagram 1. Histological results and the incidence of myasthenia gravis.

Abbreviations: PC pre-COVID-19 group, NC no-COVID-19 group, VIC vaccinated or infected COVID-19 group.

4.4.2. Immunhistochemical results

CD4 antigen expression

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) (Table 11).

	PCG	**NCG**	**VICG**
Mean	2.59	2.53	2.08
Median	3	3	2

Table 11. Immunohistochemical results of the CD4.

Abbreviations: CD cluster of differentiation, PC pre-COVID-19 group, NC no-COVID-19 group, VIC vaccinated or infected COVID-19 group.

CD8 antigen expression

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 (Table 12).

	PCG	**NCG**	**VICG**
Mean	2.18	2.53	2.5
Median	2	3	3

Table 12. Immunohistochemical results of the CD8.

Abbreviations: CD cluster of differentiation, PC pre-COVID-19 group, NC no-COVID-19 group, VIC vaccinated or infected COVID-19 group.

FOXP3 expression

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) (Table 13).

	PCG	**NCG**	**VICG**
Mean	2.41	1.12	0.92
Median	3	1	1

Table 13. Immunohistochemical results of the FOXP3

Abbreviations: FOXP3 forkhead box P3, PC pre-COVID-19 group, NC no-COVID-19 group, VIC vaccinated or infected COVID-19 group.

CD25 antigen expression

There were no significant differences between the PC, NC and VIC groups regarding the CD25 staining of thymic samples (p=0.776) (Table 14).

	PCG	NCG	VICG
Mean	1.77	1.52	1.67
Median	2	2	2

Table 14. Immunohistochemical results of the CD25.

Abbreviations: CD cluster of differentiation, PC pre-COVID-19 group, NC no-COVID-19 group, VIC vaccinated or infected COVID-19 group.

4.5 Conclusion

In the AIDS era of the 1980s, it has been suggested that the condition of AIDS patients could be improved by enhancing the thymic function, but successful antiretroviral therapy has sidelined such attempts [86, 87]. This issue has now come to the forefront of research again due to the COVID-19 infection.

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 [88]. 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.

From professional practice, the question logically arises as to how the co-expression of FOXP3 and CD25 develops, but a 'flow' cytometric test could provide an answer to this investigation. 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 [79-81], 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 [80-83], 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, 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 [84].

Limitations of study 3.

In order to follow the changes in T-cell subpopulations more precisely, it is logical to compare the occurrence of the T4 and T8 cells in the thymus with the occurrence of similar subtypes detected in the serum, but in view of the fact, that the operation of the PC group took place years ago, a blood sample representative of the immune status at the time close to the thymectomy cannot be taken. An important element of the immune response detected in a COVID-19 infection would be the examination of memory T cells. The memory cells are examined by flow cytometry, which unfortunately we did not have.

5. 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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9. ANNEX