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I.V. Paracetamol

БЕЗБЕДНА АНАЛГЕЗИЈА

менаџирање на болка кога сте загрижени за безбедноста

I.V. paracetamol за прв пат во Европа е применет во 2001 година, а денес поради неговата докажана безбедност и ефикасност е прв од избор **аналгетик и антипиретик.**

Предоперативна и Интраоперативна Аналгезија:
Предоперативна анелгезија е дефинирана како третман кој што започнува пред оперативниот зафат се со цел да се превенира воспоставувањето на централна сензибилизација на болка.
i.v. paracetamol е безбеден, добро толериран лек со докажана ефикасност како **предоперативна и интраоперативна анелгезија** за умерена до средна болка при оперативни зафати.
Голем број на клинички студии ја докажуваат ефикасноста на **i.v. paracetamol** како **преодоперативна и интраоперативна анелгезија**.

КЛИНИЧКА СТУДИЈА:
Ефект од **предоперативен i.v. paracetamol** за постоперативни аналгетски потреби кај пациенти кои се подлежни на оперативни зафати. A Sreenivasulu , R Prabhavathi, 2015
Цел: Да се утврди ефикасноста на **предоперативната употреба на 1000mg i.v. paracetamol** кај постоперативните болки и анелгетски потреби кај пациенти подлежни на хируршки зафати.
Метод: 60 пациенти беа поделени во две рандомизирани групи од по 30 пациенти.
На I. Група им беше администрирано **ампула од 1000mg i.v. paracetamol разредена 0,9%NaCl** р-ор 30 минути пред индукција **(ГРУПА П)**,
На II. Група им беше администрирано **i.v. 0,9% NaCl р-ор 100мл** 30 минути пред индукција **(ГРУПА НС)**
Сите пациенти беа индуцирани со i.v. thiopentone 5mg/kg, i.v. fentanyl 2µg/kg, i.v. vecuronium 0.1mg/kg
Постоперативниот резултат на болка беше мерен со **Визуелна Аналогна Скала (ВАС) од "0-10"**. Исто така беше забележувана и **постоперативната употреба на tramadol** како спасувачки аналгетик. Инциденцата на **постоперативно гадење и повраќање (ПОГП)** и други компликации исто така беа забележувани во пост оперативниот период.
Резултатот на постоперативната болка беше забележуван во интервали 15 мин, 30 мин, 1 час, 2 часа, и 6 часа.

Резултат:

Табела 1: *Споредба на средниот резултат на болка (ВАС) помеѓу двете групи*

Интервали	I Група П	II Група НС	*P вредност*
15 мин	2.06 ± 0.63	2.61 ± 0.56	0.0006
30 мин	2.35 ± 1.17	3.84 ± 1.55	0.0001
1 час	2.42 ± 1.12	2.87 ± 0.99	0.0989
2 часа	2.13 ± 1.06	2.52 ± 0.89	0.1219
6 часа	2 ± 0.52	2.52 ± 0.89	0.0549

Табела 2: *Споредба за потребите од tramadol помеѓу двете групи*

Интервали	I Група П	II Група НС	*P вредност*
До 1 час	4 (12.90%)	15 (50%)	0.0002
1-2 часа	3 (9.68%)	2 (6.45%)	0.64
2-6 часа	1 (3.23%)	3 (9.68%)	0.301
Вкупно	**8 (25.81%)**	**20 (64.52%)**	**0.002**

Табела3: *Споредба на ПОПГ помеѓу двете групи*

ПОГП	
I Група П	II Група НС
0	4

Заклучок: Предоперативна администрација на **1000mg i.v. paracetamol** кај пациенти подлежни на оперативен зафат обезбедува **статистички задоволителна анелегизија**, и ја **намалува постоперативната употреба на tramadol**. Оттука **1000mg i.v. paracetamol** може безбедно да се администитрира како превенција при оперативни зафати.

i.v. Paracetamol + јак опоид	МНОГУ ЈАКА БОЛКА
i.v. Paracetamol + слаб опоид	ЈАКА БОЛКА
i.v. Paracetamol + NSAID i.v. Paracetamol + rescue medicine	УМЕРЕНА БОЛКА
i.v. Paracetamol + rescue medicine	СЛАБА БОЛКА

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WHEN EARLY RECOVERY REALLY MATTERS

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CONTENT

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BETA-BLOCKERS IN SEPSIS: FACT OR MYTH?

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In the early phase of septic shock, overwhelming inflammation leads to vasodilation and capillary leakage, which decreases cardiac output due to both absolute and relative hypovolemia(1). In sepsis, the host responds to infection by activating hemodynamic, metabolic and immunological processes to attempt to restore homeostasis. The adrenergic system serves as an initial adaptive response to maintain homeostasis. Endogenous epinephrine and norepinephrine levels in serum are markedly elevated in septic patients. However, excessive catecholamine surge can cause adverse effects such as persistent tachycardia, which worsens the prognosis in patients with sepsis, and a plethora of nonhemodynamic effects(2). Besides of the increased sympathetic activity with endogenous catecholamine excess, the increasing of heart rate may be caused by the other multiple factors such as systemic and myocardial inflammation, pain, fever, hypovolemia, administration of catecholamines, autonomic dysfunction with decreased parasympathetic control of the heart, direct effects of toxins such as lipopolysaccharide and cytokines such as thromboxane A2 and prostaglandins on the myocardium, and a physiologic response to absolute or relative hypovolemia (2,3).

Patients who remained tachycardic 24 hours after fluid resuscitation and the initiation of norepinephrine infusion had a threefold higher risk of death compared to those without tachycardia (4,5). The studies indicated that patients who have been prescribed chronic beta blockers might experience better survival outcomes if they later develop sepsis and are admitted to the ICU (6). Despite several theoretical benefits of beta blockers in the early stages of sepsis, clinicians may still find the use of this therapy to be unconventional or unexpected. Sepsis-induced myocardial injury is thought to be driven by two main mechanisms: an overproduction of catecholamines and an overabundance of cytokines (1,2). Beta blockers, which affect both processes, have been suggested as potential therapies to lower mortality rates. So, the conventional view that beta blockers are inappropriate for sepsis or septic shock patients, because of cardiac suppressive effects, is now being questioned, and the use of beta -blockers in sepsis has gained increasing attention, particularly for patients with tachycardia. Decatecholaminisation is the reduction of endogenous and exogenous adrenergic stimulation (7).

Morelli et al. analyzed 45 patients with septic shock with an HR 95beats/min after at least 24 hours of resuscitation, requiring norepinephrine (NE) to maintain a MAP 65mm Hg and who were treated with a continuous esmolol infusion to achieve and maintain a target HR between 80 and 94 beats/min during their entire ICU stay (8). Out of the 45 patients included in the original study, 22 patients (48.9%) experienced a decrease in art dP/dtmax 4 hours after reducing HR with esmolol. Compared tobaseline values, the HR reduction caused a significant decrease of the CO only in the group of patients with low art dP/dtmax after esmolol administration (CO reduction from 5.0 [1.3] to 4.4 [1.0] L/min). However, in patients with high values of art dP/dtmax after esmolol administration, it was found a significantly increased SV (from 48 [12] to 67 [14] ml) with consequently maintained CO (even non-significantly increased) despite the

reduction in HR (8). The increase in stroke volume (SV) following heart rate (HR) reduction helped to maintain cardiac output. The heart rate reduction to 80–94bpm over a 4-hours period could have initially led to a decrease in cardiac output (8). However, the lower heart rate was balanced by increased ventricular filling time and volume, along with a reduction in left ventricular afterload, ultimately resulting in an increase in stroke volume, compensating for the decrease in heart rate. Notably, the left ventricular ejection fraction remained unchanged throughout the process. This change in hemodynamic can be viewed as a way to reduce myocardial workload and oxygen consumption, which in turn lowers the risk of myocardial ischemia. The reduction in arterial elastance (Ea) and the resulting improvement in ventricular-arterial coupling, combined with the reduction in myocardial workload and oxygen consumption, likely play a role in preserving myocardial efficiency, particularly in the context of established septic shock (8).

The J-land 3S study (9), multicenter, open-label, randomized controlled trial (54 hospitals) included 151 patientswith sepsis and persistent tachyarrhythmia (atrial fibrillation - AF, atrial flutter - Afl, Sinus tachycardia - ST), who were randomized to 2 groups: 76 patients who received Landiolol and standard therapy (Landiolol group) - mandatory for the first 96 h and 75 patients who received standard therapy (Control group). This study demonstrated that a higher proportion of patients in the Landiolol group achieved target heart rates compared to the control group, with a notable reduction in new arrhythmias. Specifically, 41 patients (55%) in the Landiolol group reached a heart rate of 60–94 beats/min 24 hours after enrollment, whereas only 25 patients (33%) in the control group did. This difference was statistically significant (p=0.0031). Additionally, the incidence of new arrhythmias within 7 days was significantly lower in the Landiolol group (9%) compared to the control group (25%) (p=0.015). However, there was no significant difference in 28-days mortality rates between the two groups (9).

Another clinicalmulticenter, randomized, open-label, phase 2b study published in JAMA in 2023 (The Study into the Reversal of Septic Shock with Landiolol STRESS-L) did not support the use of Landiolol in sepsis (10). The result found that administering Landiolol to sepsis patients did not decrease the SOFA score (Landiolol group 8.8 ± 3.9 vs. control group 8.1 ± 3.2 (p=0.24) but increased the 28-days mortality (37.1% in the Landiolol group vs. 25.4% in the control group, $p > 0.05$) and 90-days mortality (43.5% rates in the Landiolol group vs. 14.9% in the control group, $p > 0.05$)). More importantly, the incidence of serious adverse events in the Landiolol group (25.4%) was significantly higher than that of the control group (6.4%), with a statistical difference between the groups (p=0.006). However, there were several limitations to this study that was stopped prematurely, including: 1) the outcomes of Landiolol administration when given before or after the 24-hours norepinephrine treatment window, at different doses of norepinephrine, or in various patient sub-phenotypes; 2) the absence of data on cardiac function, either through cardiac output monitoring or echocardiography; and 3) the reduced ability to identify specific patient groups that may have either benefited from or been harmed by the intervention (10).

Hasegawa et al. performed a systematic review and meta-analysis, combining data from six randomized controlled trials with a total of 572 patients (11). Their analysis revealed that administering ultrashort-acting beta-blockers resulted in a reduction in heart rate (HR), an increase in stroke volume (SV), and no significant changes in cardiac index, mean arterial pressure, or norepinephrine dose. Moreover, the treatment was linked to a notable decrease in 28-days mortality (risk ratio 0.68 [0.54–0.85]; P< .001).

Meta-analysis of 8 out of 10 RCTs with 797 participants reported 28-days mortality outcomes (12). The results indicated that administering ultrashort-acting β- blockers (esmolol/Landiolol) to patients with sepsis who had persistent tachycardia despite initial resuscitation was significantly associated with a lower 28-days mortality rate (RR: 0.73; 95% CI: 0.57–0.93; and p<0.01). But subgroup analysis revealed that the use of esmolol in sepsis patients was significantly linked to reduced 28-days mortality (RR: 0.68; 95% CI: 0.55–0.84; and p<0.001), while there was no significant difference between the Landiolol and control groups (RR: 0.98; 95% CI: 0.41–2.34; and p=0.96). It may be that the limited sample size prevented the identification of survival benefits with Landiolol (12).

Recent findings indicate that in the early stages (< 24 hours) of septic shock, using esmolol to reduce heart rate increased the risk of hypotension and decreased the cardiac index (13). While lactate levels and microcirculatory markers remained stable, there was a reduction in most of the pro-inflammatory markers, suggesting that beta-blockade might have an immunomodulatory effect (13). Importantly, there was not registered increasing in extravascular lung water, implying that myocardial contractility, while reduced, remained adequate. This is consistent with preserved stroke volume and perfusion parameters. The results highlight that achieving optimal preload, ventricular filling, and myocardial contractility may require careful, gradual titration, potentially leading to a longer time needed to safely reach hemodynamic stability at a lower heart rate (13).

Given these findings, it is recommended to refrain from administering beta-blockers during the early stages of septic shock to minimize the risk of hindering the chronotropic response, which is crucial at this point for compensating the reduced stroke volume. The choice of short acting I.V. beta1-selective adrenergic antagonist (esmolol, Landiolol) with limited effect on blood pressure and inotropy may have advantage in aim to achieve optimal bradycardic effects.

The primary challenge is to accurately differentiate between tachycardia caused by compensatory mechanisms (due to low stroke volume) and tachycardia driven by non-compensatory factors, such as sympathetic overstimulation (9,14).This distinction is essential in determining whether controlling tachycardia will be beneficial or potentially harmful to the patient (14). While conventional hemodynamic markers and echocardiography can provide guidance on when tachycardia should not be addressed, they may not detect subtle declines in myocardial contractility that are common in septic shock, as these are often compensated by an elevated heart rate. Such myocardial dysfunction may only become noticeable after reducing the heart rate. As a result, rapid titration of beta-blockers should be avoided, and any reduction in heart rate should be carefully monitored. In practice, as heart rate increases, the rate of beta-blocker titration should be slowed accordingly. During treatment it is of utmost importance to titrate beta-blocker to the heart rate that helps to optimize hemodynamic profile in the individual patient (15). A clinically relevant drop in blood pressure or an increase in noradrenaline requirement, respectively, during short action beta-blocker titration should prompt dose reduction or discontinuation of the drug (15).

Some authors concluded that heart rate control by beta blockers may be beneficial in specific subgroups of septic patients. Until now, the only promising maker to discriminate tachycardic patients with sepsis qualified for beta-blocker use is the systolic-dicrotic notch pressure difference (16).

The difference between systolic and dicrotic pressure (SDP difference - is the result of the coupling between myocardial contractility and a given afterload) might be helpful in discriminating the origin of tachycardia. A low SDP difference in patients with septic shock with tachycardia might indicate a high risk of decompensation in case of pharmacological reduction in heart rate (16).

Echocardiographic evaluations should be performed both before and during treatment to identify any potential contraindications and monitor hemodynamic performance. Subgroups that may benefit from heart rate control in septic shock include patients with atrial fibrillation and those with preserved ejection fraction (17). This hypothesis is currently being explored in a multicenter trial (HyperBetashock, NCT04748796).

It was shown that the using of beta-blockers may be useful particularly in patients with LV hyperkinesia and well-resuscitated phenotype, but not useful or detrimental in left ventricular (LV) systolic dysfunction, still hypovolemic patients, and in right ventricular failure (18).

Left intraventricular flow obstruction (IVO) is typically associated with asymmetric hypertrophic cardiomyopathy. Dynamic IVO can also occur following aortic stenosis ormitral valve repair, particularly if the positioning of the mitral prosthesis interferes with the left ventricular outflow tract (LVOT). Obstruction may also be observed in patients at risk for hypovolemia, tachycardia, or those exposed to catecholamines, as well as in individuals with a narrowed LVOT, a small LV lumenand LV hyperkinesia. In these cases, beta-blocker therapy may be considered, provided the patient has received adequate fluid resuscitation (19).

In conclusion, beta-blocker therapy could be advantageous for septic patients, but it requires careful consideration. Proper patient selection is crucial, with short-acting beta-blockers being the preferred option. Echocardiography plays an important role in identifying patients who might not tolerate beta-blocker treatment.

References:

1. Evans L, Rhodes A, Alhazzani W, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. Crit Care Med. 2021;49(11):e1063–e1143.
2. Andreis DT, Singer M. Catecholamines for inflammatory shock: a Jekyll-and-Hyde conundrum. Intensive Care Med. 2016 Sep;42(9):1387-97.
3. Martin-Loeches I, Singer M, Leone M. Sepsis: key insights, future directions, and immediate goals. A review and expert opinion Intensive Care Med 2024; 50:2043-2049.
4. Hoke RS, Müller-Werdan U, Lautenschläger C, et al. Heart rate as an independent risk factor in patients with multiple organ dysfunction: a prospective, observational study. Clin Res Cardiol. 2012 Feb;101(2):139-47.
5. Unger M, Morelli A, Singer M, et al. Landiolol in patients with septic shock resident in an intensive care unit (LANDI-SEP): study protocol for a randomized controlled trial. Trials. 2018 Nov 19;19(1):637.
6. Macchia A, Romero M, Comignani PD, et al. Previous prescription of β-blockers is associated with reduced mortality among patients hospitalized in intensive care units for sepsis. Crit Care Med. 2012 Oct;40(10):2768-72.

7. Rudiger A, Singer M. Decatecholaminisation during sepsis. Crit Care 2016; 20, 309.

8. Morelli A, Singer M, Ranieri VM, et al. Heart rate reduction with esmolol is associated with improved arterial elastance in patients with septic shock: a prospective observational study. Intensive Care Med. 2016 Oct;42(10):1528-1534.

9. Kakihana Y, Nishida O, Taniguchi T, et al. Efficacy and safety of landiolol, an ultra-short-acting β1-selective antagonist, for treatment of sepsis-related tachyarrhythmia (J-Land 3S): a multicentre, open-label, randomised controlled trial. Lancet Respir Med. 2020 Sep;8(9):863-872.

10. Whitehouse T, Hossain A, Perkins GD, et al. Landiolol and Organ Failure in Patients With Septic Shock: The STRESS-L Randomized Clinical Trial. JAMA. 2023 Nov 7;330(17):1641-1652.

11. Hasegawa D, Sato R, Prasitlumkum N, et al. Effect of Ultrashort-Acting β-Blockers on Mortality in Patients With Sepsis With Persistent Tachycardia Despite Initial Resuscitation: A Systematic Review and Meta-analysis of Randomized Controlled Trials. Chest. 2021 Jun;159(6):2289-2300.

12. Huang P, Liu F, Hu X, et al. Effect of ultrashort-acting β-blockers on 28-day mortality in patients with sepsis with persistent tachycardia despite initial resuscitation: a meta-analysis of randomized controlled trials and trial sequential analysis. Front Pharmacol. 2024 Jun 20;15:1380175.

13. Levy B, Fritz C, Piona C, et al. Hemodynamic and anti-inflammatory effects of early esmolol use in hyperkinetic septic shock: a pilot study. Crit Care. 2021 Jan 7;25(1):21.

14. Morelli A, Sanfilippo F. Ultrashort-Acting β-Blockers: Are We on the Right Path? Chest. 2021 Jun;159(6):2139-2140.

15. Volpicelli G, Balik M, Georgopoulos D. Echography is mandatory for the initial management of critically ill patients: no. Intensive Care Med. 2014 Nov;40(11):1766-1768.

16. Morelli A, Romano SM, Sanfilippo F et al. Systolic-dicrotic notch pressure difference can identify tachycardic patients with septic shock at risk of cardiovascular decompensation following pharmacological heart rate reduction. Br J Anaesth 2020; 125:1018-1024.

17. Rehberg S, Duska F. Heart rate control in septic shock—"every journey begins with a first step" (Confucius). Intensive Care Med 2025; 51: 222-223.

18. Geri G, Vignon P, Aubry A, et al. Cardiovascular clusters in septic shock combining clinical and echocardiographic parameters: a post hoc analysis. Intensive Care Med. 2019 May;45(5):657-667.

19. Chauvet JL, El-Dash S, Delastre O, et al. Early dynamic left intraventricular obstruction is associated with hypovolemia and high mortality in septic shock patients. Crit Care. 2015 Jun 17;19(1):262.

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ASSOCIATION BETWEEN VITAMIN D DEFICIENCY AND INSULIN RESISTANCE IN EARLY PREGNANCY: A CROSS-SECTIONAL STUDY

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Abstract

Introduction: Vitamin D is crucial in the metabolism of calcium and phosphorus, as well as in bone health, while also contributing to numerous other bodily functions. Vitamin D deficiency in pregnant women is very common worldwide. It is associated with an increased risk of preeclampsia, gestational diabetes mellitus and cesarean section. Consequences in the newborn are most often associated with low birth weight, risk of neonatal hypocalcemia, asthma, and/ or type 1 diabetes mellitus. A lack of vitamin D during pregnancy is linked to various metabolic issues, including insulin resistance. It is considered that increased body weight has a negative effect on the concentration of vitamin D. Deficiency of 25-hydroxyvitamin D has long been considered a risk factor for glucose intolerance and most likely 1,25-dihydroxyvitamin D has a role in the regulation of insulin secretion.

Objective: To investigate how a deficiency in vitamin D impacts the onset of insulin resistance in pregnant women.

Material and Methods: A cross-sectional clinical study was conducted in the University Clinic for Endocrinology, Diabetes and Metabolic Diseases, Skopje, from March 2022 to March 2023 with 55 pregnant women in the first trimester of pregnancy. According to vitamin D values, the sample subjects were divided into three groups: a) Group 1: <20ng/ml; b) Group 2: 20-44ng/ml; and c) Group 3: >44ng/ml. We analyzed the level of insulinemia, glycemia and homeostatic model assessment for insulin resistance (HOMA IR) in the three groups.

Results: Among 55 pregnant women assessed in their first trimester, 30 (54.54%) showed a vitamin D deficiency (below 20ng/ml). Nineteen patients (34.54%) had normal vitamin D levels (ranging from 20 to 44ng/ml), while 6 (19.91%) had elevated levels (above 44ng/ml). In the group with vitamin D deficiency, the average HOMA IR value was higher at 3.14±1.59, compared to an average of 2.57±1 in the group with normal vitamin D levels.

Conclusion: A shortage of vitamin D during the first trimester of pregnancy is linked to increased insulin resistance, which can complicate metabolic health. Therefore, adequate supstituion of vitamin D during pregnancy is necessary for mother's and offspring's wellbeing.

Key Words: *glycemia; insulin resistance*; *pregnancy; vitamin D.*

Introduction

Vitamin D plays an essential part in regulating calcium and phosphorus metabolism and maintaining bone health, while also contributing to a wide range of other bodily processes. A lack of vitamin D is frequently observed during pregnancy and has been connected to heightened risks of complications such as preeclampsia, gestational diabetes, early delivery, cesarean section, and delivering a baby smaller than expected for its gestational age. For newborns, this deficiency is often tied to issues like low birth weight, a greater chance of neonatal hypocalcemia, asthma and the potential development of type 1 diabetes. Additionally, there appears to be a link between conditions like attention deficit disorder and autism spectrum development (1). Vitamin D is a steroid hormone that plays a role in regulating body homeostasis, including cardiovascular function. A connection exists between low vitamin D levels and a rise in cardiovascular risk factors. It's believed that providing vitamin D supplements could enhance outcomes for individuals with heart-related conditions. Beyond its role in bone health, vitamin D exhibits anti-inflammatory properties and influences various systems in the body. It has been linked to the emergence of infectious diseases, autoimmune disorders, cardiometabolic conditions and the initiation of certain cancers (2). Insulin resistance is a multifaceted condition that contributes to the development of cardiovascular risk factors. As such, it is viewed as either a direct outcome or an indirect result of insufficient vitamin D levels (2). Vitamin D deficiency can be blamed on the same pathogenetic mechanisms that lead to the development of insulin resistance. Insulin resistance can be improved with proper diet and physical activity. Diet and exercise are believed to be linked to higher vitamin D levels, and addressing insulin resistance is key to boosting those levels. This underscores the importance of preventing vitamin D deficiency in pregnant women. At present, a vitamin D level of approximately 30ng/mL is advised during pregnancy. Vitamin D, a fat-soluble steroid prohormone, serves endocrine, autocrine and paracrine roles, and exists in forms like ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3). Its primary metabolites, produced through hydroxylation, include calcidiol (25(OH)D) and calcitriol (1,25-dihydroxyvitamin D3, or 1,25(OH)2D) (3).

Vitamin D acts as a signaling molecule and plays a role in regulating the transcription of about 3% of the human genome. In the bloodstream, it binds to the vitamin D-binding protein, which carries it to the liver, where it is transformed into 25(OH)D by the enzyme 25-hydroxylase. This compound is then converted in the kidneys into 1,25(OH)2D, the active form of vitamin D, through the action of 25-hydroxyvitamin D-1 alpha-hydroxylase. Additionally, vitamin D has been found to interact with the insulin receptor gene, suggesting its involvement in the transcriptional regulation of insulin (4).

Glutathione plays a vital role in managing vitamin D levels by aiding in its transformation into active metabolites. Vitamin D, in turn, boosts glutathione levels, helping to lower oxidative stress. Consequently, a lack of glutathione is tied to insulin resistance, a common feature in metabolic disorders like obesity and diabetes. In individuals with type 2 diabetes, vitamin D supports pancreatic beta-cell function, with calcitriol acting as a signaling molecule that interacts directly with beta-cell receptors to enhance their performance. It also influences insulin release by controlling calcium channel activity, improves insulin sensitivity by promoting insulin receptor expression, and activates the peroxisome proliferator receptor delta. Furthermore, vitamin D helps curb chronic inflammation by suppressing inflammatory cytokines linked to insulin resistance.

In humans, vitamin D is primarily activated through skin exposure to sunlight, consumption of foods high in vitamin D2 and D3, or supplementation. However, determining an ideal 25(OH) D concentration remains debated, with no universally agreed-upon thresholds for optimal vitamin D levels. Most of the tissues and organs possess vitamin D receptors, highlighting its involvement in numerous biological processes. During pregnancy, a deficiency in vitamin D has been connected to various metabolic issues, including insulin resistance. Pregnancy naturally increases insulin resistance, a key indicator of gestational diabetes, and excessive weight gain during this period is believed to further reduce vitamin D levels (5).

Objectives

The objectives of the study were to determine the effect of vitamin D deficiency during pregnancy on the development of insulin resistance.

Materials and Methods

A cross-sectional clinical study was conducted at the University Clinic of Endocrinology, Diabetes and Metabolic Diseases, Skopje, from March 2022 to March 2023. The study included 55 patients in the first trimester of pregnancy. A detailed medical history for each patient was used to obtain data on demographic characteristics, gestational week of pregnancy and vitamin D values, fasting insulinemia, fasting glycemia, and an insulin resistance value calculation homeostatic model assessment for insulin resistance (HOMA IR) was performed.

According to vitamin D values, the sample subjects were divided into three groups: a) Group 1: <20ng/ml; b) Group 2: 20-44ng/ml; and c) Group 3: >44ng/ml. We analyzed the level of insulinemia, glycemia and HOMA IR in the three groups.

Statistical Processing

The data was processed in the SPSS software package, version 22.0 for Windows. Qualitative series were analyzed with ratios, proportions and rates, and quantitative series with measures of central tendency and measures of dispersion. The Shapiro-Wilk W test was used to determine the regularity of the frequency distribution of the variables examined. The Pearson Chi-square test was used to determine the association between certain traits in the groups of respondents. The Pearson correlation coefficient, as well as Partial correlations were used to determine the association between the numerical variables of vitamin D and HOMA IR with no adjustment for age and gestational week of pregnancy. Independent numerical parameters were compared with the Kruskal-Wallis H test. To determine statistical significance, a two-sided analysis with a significance level of $p<0.05$ was used.

Results

The study enrolled 55 patients in the first trimester of pregnancy, with a median gestation week of the whole sample of 9.76 ± 1.89, with a minimum of 6 and maximum of 13 weeks of gestation,

and with 50% of patients below 10 weeks of gestation for Median IQR=10 (8-11). The mean age of the patients in the sample was 30±4.66 years with a min/max age of 20/39 years. For 75% of respondents in the sample, the age was less than 34 years for a median IQR of 31 (29-34).

The mean vitamin D value in the entire sample of respondents was 22.87±14.50ng/mL, with a minimum value of 3ng/mL and 70ng/mL, respectively. In 50% of pregnant women, the vitamin D value was less than 18.12ng/mL. According to vitamin D values, the sample subjects were divided into three groups: a) Group 1: <20ng/ml in 30 (54.54%); b) Group 2: 20-44ng/ml in 19 (34.54%); and c) Group 3: >44ng/ml in 6 (19.91%). The mean vitamin D value for the three groups was consequentially 13.66±3.98 vs. 26.53±4.42 vs. 57.35±11.11ng/ml, a significant difference between the groups with the expected lowest mean value in Group 1 and highest in Group 3 (Kruskal-Wallis H test: Chi-square (2) = 42.956; p = 0.00001) (Table 1).

In pregnant women in the three vitamin D groups, the mean insulin was 14.72±7.16 in Vit D Group 1, 12.81±5.12 in Vit D Group 2, and 14.79±6.28 in Vit D Group 3. In 50% of the subjects in the three groups, insulin values were consequentially < 14.4 vs. 13.5 vs. 13.7 with no significant intergroup difference (Kruskal-Wallis H test: Chi-square (2)=0.478; p=0.7873) (Table 1).

Table 1. Intergroup comparison of vitamin D by selected parameters.

Parameters	Values Obtained						
	(No.)	Mean± SD	(Min/Max)	Percentiles			[1]p
				25th	50th (Median)	75th	
Vitamin D (ng/ml)							
Vit D - Group 1	30	13.66±3.98	3/ 19.7	11.7	14	17.1	$X^2(2)=42.956$; p=0.00001*
Vit D - Group 2	19	26.53±4.42	20.8/ 37.5	22.3	26	30.1	
Vit D - Group 3	6	57.35±11.11	44.5/ 7	45.9	56.9	70	
Insulin							
Vit D - Group 1	30	14.72±7.16	5.2/ 40.9	10.1	14.1	17.9	$X^2(2)=0.478$; p=0.7873
Vit D - Group 2	19	12.81±5.12	2.4/ 20.2	9.3	13.5	16.4	
Vit D - Group 3	6	14.79±6.28	7.9/ 24.7	9.5	13.7	19.1	
Glycemia							
Vit D - Group 1	30	4.88±0.55	3.4/ 5.8	4.6	5	5.2	$X^2(2)=5.500$; p=0.0639
Vit D - Group 2	19	4.59±0.40	4/ 5.4	4.2	4.6	4.9	
Vit D - Group 3	6	4.88±0.31	4.5/ 5.3	4.6	4.9	5.1	
HOMA IR							
Vit D - Group 1	30	3.14±1.59	0.9/ 8.9	2.2	3.2	3.6	$X^2(2)=1.726$; p=0.4220
Vit D - Group 2	19	2.57±1.15	0.5/ 4.7	1.9	2.6	3.1	
Vit D - Group 3	6	3.18±1.47	1.7/ 5.4	1.9	2.8	4.5	

Vit D - Group 1: <20ng/ml; Vit D - Group 2: 20-44ng/ml; and Vit D - Group 3: >44ng/ml
[1]Kruskal-Wallis H test *significant for p<0.05

Analysis in terms of glycemic levels indicated a marginally lower average glycemic value in Vitamin D-Group 2 of 4.59±0.40 compared to Vitamin D-Group 1 and Vitamin D-Group 3, where the mean value was consequentially 4.88±0.55 vs. 4.88±0.31 (Kruskal-Wallis H test: Chi-square (2) = 5.50; p = 0.0639) (Table 1).

The mean HOMA IR was indistinctly low in Vitamin D-Group 2 of 2.57±1.15 compared to Vitamin D-Group 1 and Vitamin D-Group 3, where the mean HOMA IR was consequentially 3.14±1.59 vs. 3.18±1.47 (Kruskal-Wallis H test: Chi-square (2) = 1.726; p=0.4330) (Table 1).

The majority of patients from the three Vitamin D groups had a HOMA IR≥2.5 and 22 (73.33%) in Vitamin D-Group 1; 11 (61.11%) in Vitamin D-Group 2; and 4 (66.67%) in Vitamin D-Group 3 (Table 2). No significant association was found between patients belonging to any of the three vitamin D groups and HOMA IR values <2.5 and ≥2.5 (X2=0.7898; df=2; p=0.6737).

Table 2. Analysis of vitamin D and HOMA IR groups.

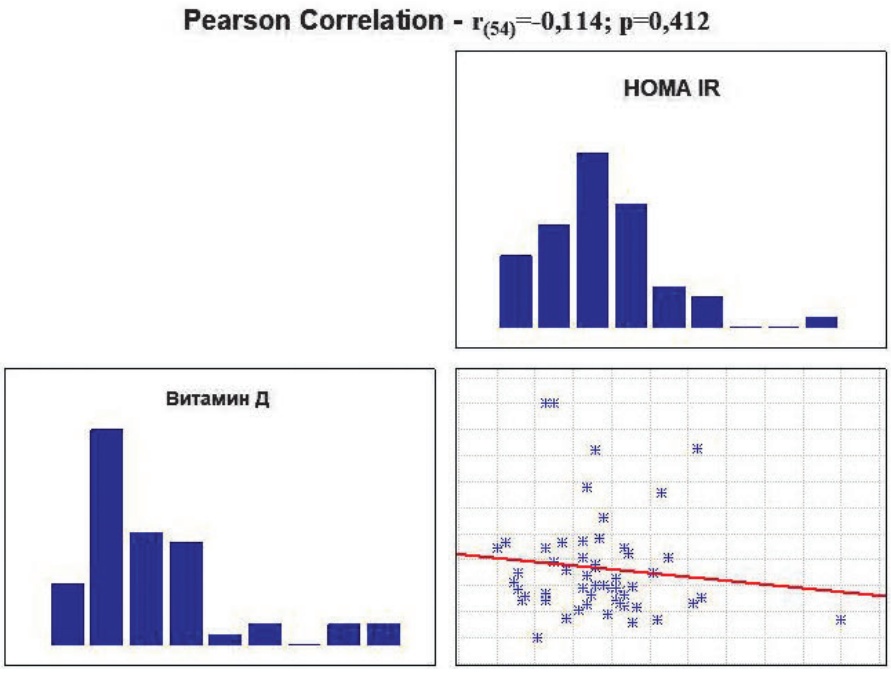
Vitamin D	HOMA IR		Total	[1]p
	HOMA IR <2.5	HOMA IR ≥2.5		
Groups				
Vit D - Group 1	8 (26.67%)	22 (73.33%)	30 (55.56%)	X^2=0.7898; df=2; p=0.6737
Vit D - Group 2	7 (38.89%)	11 (61.11%)	18 (33.33%)	
Vit D - Group 3	2 (33.33%)	4 (66.67%)	6 (11.11%)	
Vit D - Group **1**: <20ng/ml; Vit D - Group 2: 20-44ng/ml; и Vit D - Group 3: >44ng/ml X^2 = Pearson Chi-square test; *significant for p<0.05				

An analysis was performed on the correlation of vitamin D levels with HOMA IR levels without and with gender-adjusted gestational weeks of study pregnant women (Table 3 and Graph 1).

Table 3. Correlation between vitamin D and HOMA IR without and with adjustment for age and gestation week.

Option	HOMA IR		
	unajusted[1]	ajusted[2]	ajusted[3]
Vitamin D	r (54)=-0.114; p=0.412	r (54)=-0.795; p=0.571	r (54)=-0.095; p=0.500
[1]Pearsons moment order correlations [2]Partial correlations – adjusted for age; [3]Partial correlations - Adjusted for gestational weeks *significant for p<0.05			

Correlation analysis revealed the presence of a nondistinct linear negative correlation between vitamin D and HOMA IR levels (r(54)=-0.114; p=0.412) with increasing vitamin D levels and nondistinctly decreasing HOMA IR levels. No significant difference was found in the strength of the correlation between vitamin D levels and HOMA IR levels before and after age-related adjustment and gestational age (Table 3 and Graph 1).



Graph 1. Correlation between Vitamin D and HOMA IR without and with Adjustment.

Discussion

In our study of the 55 patients evaluated in the first trimester, 30 (54.54%) were deficient in vitamin D (<20ng/ml). Normal vitamin D values (20–44ng/ml) were observed in 19 patients (34.54%), while higher vitamin D levels (> 44ng/ml) were found in 6 patients (19.91%). In the first group of patients with vitamin D deficiency, a higher average insulin value of 14.72 was observed compared to the average insulin value of 12.81 in the normal group of patients with vitamin D. In terms of glycemia, it indicated a marginally lower average glycemic value in Vitamin D-Group 2 of 4.59±0.40 compared to Vitamin D-Group 1 and Vitamin D-Group 3, where the mean value was consequentially 4.88±0.55 vs. 4.88±0.31.

The study by Maghbooli et al., which analyzed 741 pregnant women, found that the prevalence of vitamin D deficiency was found in 70.6% of pregnant women. The prevalence of severe vitamin D deficiency (<12.5nmol/L) in patients with gestational diabetes was higher than in patients without gestational diabetes. They found a positive correlation between vitamin D and insulin sensitivity. Vitamin D deficiency could be a sign of insulin resistance and a higher probability of gestational diabetes during the pregnancy (5).

Christoph and colleagues, in a study involving 1,382 pregnant women, reported that 73.23% of the pregnant women are with a deficit of vitamin D. A severe deficit of vitamin D (vitamin D levels below 25nmol/L) was found in 34.2% of all pregnant women. They found an association between low vitamin D, increased insulin levels and gestational diabetes (6).

In our study, the vitamin D-deficient group had a higher average HOMA IR of 3.14±1.59 compared to the normal vitamin D group with a HOMA IR of 2.57±1.15, similar to the previous cited studies.

Diseases associated with insulin resistance are becoming all too common. Vitamin D deficiency has been blamed at the molecular level as one of the risk factors leading to insulin resistance (7).

Prevention from cardiometabolic diseases, cancer development and anti-inflammatory properties are the main extra skeleton activity of vitamin D (8). Supplementation with vitamin D during pregnancy in a woman with a low level of vitamin D can improve the growth of the fetus and reduce the risks for small for gestational age, preterm birth, preeclampsia and gestational diabetes. The link between vitamin D deficiency and adverse maternal outcomes is very common, like high blood pressure during pregnancy, preterm delivery, cesarian section recurrent pregnancy loss and postpartum depression (9).

Mothers who have sufficient levels of vitamin D have offspring with less attention deficit, hyperactive disorders and autism (10). A severe deficit of vitamin D in the pregnant woman has been associated with disordered skeletal homeostasis, congenital rickets and fractures in the newborn (11).

According to the National Institute for Health and Clinical Excellence, United Kingdom, the daily dosage of Vitamin D in all pregnant women should be 400IU (12.) According to the Endocrine Society the average dose is 1500–2000 IU (13), and 2000 IU by the Canadian Society (14).

Emerging research has highlighted additional consequences of vitamin D deficiency during pregnancy that extend beyond those previously discussed. One significant area of concern is the potential impact on the epigenetic regulation of the developing fetus. Vitamin D plays a crucial role in the regulation of gene expression through epigenetic mechanisms, such as DNA methylation. Deficiencies in vitamin D during pregnancy have been associated with alterations in these epigenetic marks, which can influence fetal development, and have lasting effects on offspring health. Notably, studies have demonstrated that maternal vitamin D deficiency can lead to changes in DNA methylation patterns that persist across multiple generations, affecting both somatic and germline tissues. These epigenetic modifications have been linked to variations in body weight and metabolic function in the offspring (15, 16). Adequate vitamin D levels are essential for proper neurodevelopment. Emerging evidence suggests that maternal vitamin D deficiency may be associated with an increased risk of neurodevelopmental disorders in offspring, such as schizophrenia. Vitamin D is essential for the normal development of the nervous system, and its deficiency during pregnancy can cause prenatal neurodevelopmental defects, influencing neurotransmission and brain function (17). Vitamin D is known to modulate the immune system, and its deficiency during pregnancy may have implications for the immune development of the fetus as well. While specific studies on this aspect are limited, it is plausible that inadequate maternal vitamin D levels could influence the neonatal immune response, potentially affecting susceptibility to infections and the development of autoimmune conditions later in life (18).

Conclusion

General screening for vitamin D deficiency, the timing of supplementation before conception, and personalized vitamin D dosing appear essential, possibly resulting in better maternal health and advantages for children. Providing vitamin D supplements to those who are deficient is essential not only to address this issue but also to improve overall pregnancy outcomes, potentially reducing related risks and supporting maternal and fetal wellbeing.

References:

1. Pérez-López, Faustino R.; Pilz, Stefan ; Chedraui, Peter ; Vitamin D supplementation during pregnancy: an overview. Current Opinion in Obstetrics and Gynecology, 2020 October, 32(5):p 316-321.
2. Trimarco V, Manzi MV, Mancusi C, et al. Insulin Resistance and Vitamin D Deficiency: A Link Beyond the Appearances. Front Cardiovasc Med. 2022; Mar 17;9:859793.
3. Contreras-Bolívar V, García-Fontana B, García-Fontana C, Muñoz-Torres M.; Mechanisms Involved in the Relationship between Vitamin D and Insulin Resistance: Impact on Clinical Practice. Nutrients. 2021 Oct 1;13(10):3491.
4. Bikle DD. Vitamin D: Production, Metabolism, and Mechanisms of Action. [Updated 2021 Dec 31]. In: Feingold KR, Anawalt B, Blackman MR, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-.
5. Maghbooli Z, Hossein-Nezhad A, Karimi F, Shafaei AR, Larijani B. Correlation between vitamin D3 deficiency and insulin resistance in pregnancy. Diabetes Metab Res Rev. 2008 Jan-Feb;24(1):27-32.
6. Christoph P, Challande P, Raio L, Surbek D. High prevalence of severe vitamin D deficiency during the first trimester in pregnant women in Switzerland and its potential contributions to adverse outcomes in the pregnancy. Swiss Med Wkly. 2020 May 28;150.
7. Argano C, Mirarchi L, Amodeo S, Orlando V, Torres A, Corrao S. The Role of Vitamin D and Its Molecular Bases in Insulin Resistance, Diabetes, Metabolic Syndrome, and Cardiovascular Disease: State of the Art. Int J Mol Sci. 2023 Oct 23;24(20):15485.
8. Szymczak-Pajor I, Śliwińska A. Analysis of Association between Vitamin D Deficiency and Insulin Resistance. Nutrients. 2019 Apr 6;11(4):794.
9. Mithal A, Kalra S. Vitamin D supplementation in pregnancy. Indian J Endocrinol Metab. 2014 Sep;18(5):593-6.
10. Pérez-López FR, Pilz S, Chedraui P. Vitamin D supplementation during pregnancy: an overview. Curr Opin Obstet Gynecol. 2020 Oct;32(5):316-321.
11. Pawley N, Bishop NJ. Prenatal and infant predictors of bone health: the influence of vitamin D. Am J Clin Nutr 2004;80:1748S–51S.
12. Godel JC. Postition statement vitamin D supplementation: Recommendations for Canadian mothers and infants. [Last accessed on 2014 Mar 15].
13. Marya RK, Rathee S, Lata V, Mudgil S. Effects of vitamin D supplementation in pregnancy. Gynecol Obstet Invest. 1981;12:155–61.
14. Marya RK, Rathee S, Manrow M. Effect of calcium and vitamin D supplementation on toxaemia of pregnancy. Gynecol Obstet Invest. 1987;24:38–42.
15. Xue J, Gharaibeh RZ, Pietryk EW, Brouwer C, Tarantino LM, Valdar W, Ideraabdullah FY. Impact of vitamin D depletion during development on mouse sperm DNA methylation. Epigenetics. 2018;13(9):959-974. doi: 10.1080/15592294.2018.1526027. Epub 2018 Oct 21.
16. Xue J, Schoenrock SA, Valdar W, Tarantino LM, Ideraabdullah FY. Maternal vitamin D depletion alters DNA methylation at imprinted loci in multiple generations. Clin Epigenetics. 2016 Oct 12;8:107. doi: 10.1186/s13148-016-0276-4.

17. Cherniack EP, Levis S, Troen BR. Hypovitaminosis D: a widespread epidemic. Geriatrics. 2008 Apr;63(4):24-30. PMID: 18376898.

18. Martens PJ, Gysemans C, Verstuyf A, Mathieu AC. Vitamin D's Effect on Immune Function. Nutrients. 2020 Apr 28;12(5):1248. doi: 10.3390/nu12051248. PMID: 32353972; PMCID: PMC7281985.

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ECHOCARDIOGRAPHIC ESTIMATION OF PULMONARY CAPILLARY WEDGE PRESSURE LEVELS IN MECHANICALLY VENTILATED VERSUS SPONTANEOUSLY BREATHING PATIENTS WITH PULMONARY CONTUSIONS

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Abstract

Pulmonary contusions mediated immunological lung injury characterized with interstitial involvement, as well as therapeutically applied positive pressures over the already damaged alveoli could lead to elevation of the pressures inside pulmonary circulation. Fifty patients were included in the study and divided into two groups, whereby 26 were mechanically ventilated while 24 were spontaneously breathing patients. In all patients Pulmonary Capillary Wedge Pressure (PCWP) was measured non-invasively using echocardiography. Measurements of Left Atrial Pressure (LAP) were made 24 hours after admission, on the 7th and 14th day of admission in the ICU. PCWP was calculated using the LAP values and the Nagueh formula (LAPx1.24+1.98). Lung ultrasound was used for the measurement of severity of the lung injury according to the BLUE Protocol. Mean values for PCWP were calculated in both groups and the difference was examined using the Student T Test. It was found that the values for PCWP did not differ significantly between the groups when measured 24 hours and 7 days after admission and mechanical ventilation initiation (t = -0.11 and 1.13 for value for p 0.45 and 0.13),but were significantly higher in mechanically ventilated patients after 14 days of mechanical ventilation commencement (mean 10.52 vs. 7.88, t=1.89 for p of 0.03). Lung injury in patients with pulmonary contusions, as well as inflammation during the process of damage and repair, involves interstitial changes which can lead to affection of small pulmonary vessels as well. Therapeutically applied positive pressure ventilation and alveolar distension could impair blood flow through small pulmonary capillaries and vessels. When both combined, they can lead to elevation of small circulation pressures. Mechanical ventilation in patients with pulmonary contusions is associated with higher PCWP after 14 days of positive pressure ventilation in comparison to spontaneously breathing patients. Duration of mechanical ventilation is associated with higher levels of PCWP.

Key Words: *Mechanical ventilation; PCWP; Pulmonary contusions.*

Introduction

Pulmonary contusions are frequently met in polytraumatized patients demanding admission in the Intensive Care Unit. Approximately 25-80% of patients with chest trauma develop pulmonary contusion which is considered as a serious morbidity and mortality predictor (1). After trauma occurrence which implies kinetic energy transfer over the lung, according to Kurt A. et al., the second attack of the lung is mediated by the exaggerated immunologic response involving cytokines and proinflammatory mediators release leading to generalized lung injury (2). Therefore, inflammation mediated capillary leak leads to higher vascular permeability and leucocyte migration outside the pulmonary vasculature into interstitial space and into alveoli impairing gas exchange. Impaired gas exchange by itself leads to elevation of pulmonary vascular resistance, and in some cases, it demands mechanical ventilation with positive pressure which additionally can have negative impact over pulmonary circulation. According to abovementioned facts we assume that mechanical ventilation in patients with pulmonary contusions can have deleterious effects over the heart and systemic hemodynamics. Therefore, left atrial pressure (LAP) was assessed using echocardiography in both mechanically ventilated and spontaneously breathing patients in order to calculate Pulmonary Capillary Wedge Pressure (PCWP) since LAP can be measured using echocardiography and PCWP can be calculated using equitation of Nagueh with high accuracy (3,4).

Aim of the Study

The aim of this study is to reveal does PCWP in patients with pulmonary contusions differ significantly in between mechanically ventilated versus spontaneously breathing patients. It is expected that the results of this study will help in understanding does mechanical ventilation when applied possesses additional negative effect over the already injured lungs. Since lung injury by itself could be related to changes in PCWP, we have measured Lung Ultrasound Severity Score (LUSS) in order to quantify the severity of lung contusion and to examine the possible relationship between LUSS and PCWP.

Material and Methods

In total, 50 polytraumatized patients with pulmonary contusions were included in this study. All patients that were included in the study had signed informed consent by a family member since some of them were sedated and unconscious. All patients that we had a signed informed consent for and were older than 18 years of age fulfilling the inclusion criteria were included in the study. Pulmonary contusions without pneumothorax were detected with computed tomography which has been evaluated by a radiologist prior to patient's admission in the ICU. Patients who were previously resuscitated, with already known pulmonary hypertension or heart pathology, with pneumothorax or pregnant women were excluded from the study. The study was conducted at the Clinical Campus "Mother Theresa" in Skopje at the University Clinic for Traumatology, Orthopedics, Anesthesia, Reanimation, Intensive Care and Emergency department – Skopje at the Department of Anesthesiology, Reanimation and Intensive Care. Conduction of this study was approved by the Ethical Committee for Human Research of Medical Faculty – Skopje at "Ss Cyril and Methodius" University – Skopje on 1st of February 2023 with number of approval 03-300/3. All patients were divided regarding the need for mechanical ventilation into two groups,

a mechanically ventilated and spontaneously breathing group. Echocardiographic examination was performed in all participants 24 hours after admission, 7 days after admission and 14 days after admission. In order to calculate Pulmonary Capillary Wedge Pressure (PCWP), it was examined the left ventricle according to the Recommendations for Cardiac Chamber Quantification by Echocardiography in Adults published by the American Society of Echocardiography and the European Association of Cardiovascular Imaging (5). Firstly, transmitral pulse wave doppler has been used in order to detect transmitral E/A (Figure 1A). Afterwards, with usage of Tissue Doppler, e' was detected and measured using Pulse Wave Doppler (Figure 1B). Measured values for E and e' when divided as in the equitation E/e', gave us the value for LAP. Consequently, LAP was used for calculation of PCWP according to the Nagueh equitation (3,4). PCWP is equal to LAPx1.24+1.98 and it has been already proven that can be substitute of invasively measured PCWP without significant difference and with satisfying accuracy (3,4). From the measured values, mean PCWP was calculated in both mechanically ventilated versus spontaneously breathing patients, while the difference between them was examined using the Student T Test.

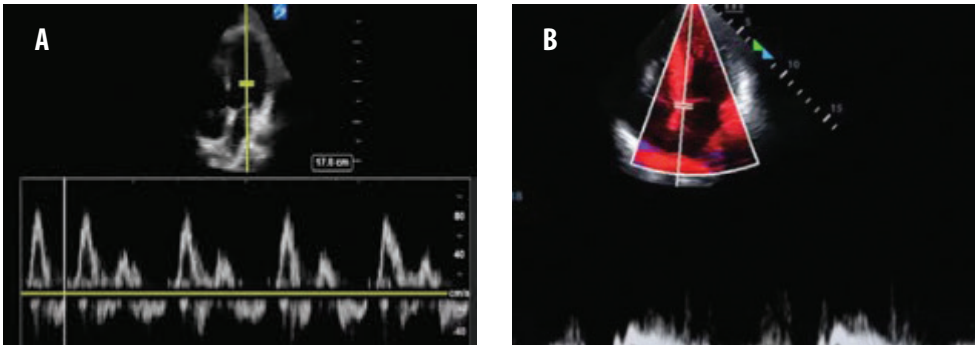

Figure 1. Transmitral Pulse Wave Doppler whit tracing of E and A waves (1A) and measurement of Septal e' with tissue doppler and Pulse wave Doppler (1B).

At the same examining points when LAP and PCWP were measured, LUS Score was measured, as well. LUS Score was measured by performing an ultrasonographic examination of both lungs divided in total 12 segments or 6 of them in each lung according to Lichtenstein's Protocol (6). Upper anterior, lateral and posterior and lower anterior, lateral and posterior segment in each lung were scanned with curvilinear probe in order to assess the severity of lung contusion. Normal aeration was seen as the presence of A-lines which are parallel to the pleural line and were scored with 0 points (Figure 2A). The existence of interstitial syndrome because of capillary leak was recognized when 3-5 B-lines were seen at any part of the lung scoring them as 1 point (Figure 2B). When more than 5 B-lines were detected or multiple B-lines were present, the existence of alveolo-interstitial syndrome was established and scored with 2 points (Figure 2C). The presence of dense consolidations with a hepatic or tissue-like structure were scored with 3 points (Figure 2D). All scanned segments in total were referred to as Lung Ultrasound Severity Score (LUSS) which could vary from 0-36 depending on the injury heaviness. Mean LUS Scores have been calculated for all examining points, and we compared them in between mechanically ventilated versus spontaneously breathing patients. In order to understand does the severity of lung injury exemplified by LUSS could have an impact over the PCWP correlation between those two variables 24 hours after admission, 7 days and 14 days after admission, it was assessed with Pearson's Correlation Test.

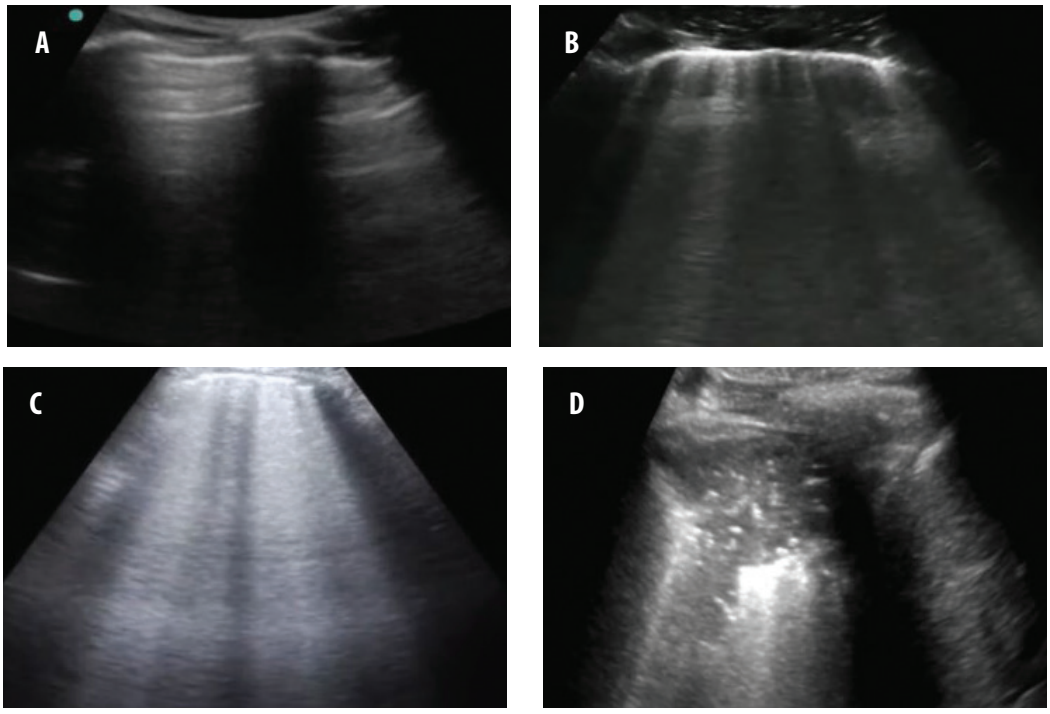

Figure 2. Ultrasonographic findings of pulmonary ultrasound: **Figure 2A.** Presence of A-lines showing good aeration of the lung scored with 0 points; **Figure 2B.** Presence of B-lines scored with 1 point; **Figure 2C.** Presence of multiple B-lines with their confluence scored with 2 points and **Figure 2D.** Pulmonary consolidation scored with 3 points according to LUSS.

Results

Among the 50 polytraumatized patients that were included in the study, 26 of them (52%) were mechanically ventilated, versus 24 (48%) who were in the spontaneously breathing group not needing mechanical ventilation. According to the values for measured LAP and calculated PCWP, we did not find statistically significant difference between groups 24 hours after admission (Mean PCWP: 8.09 versus 9.12; Student T-test: t=-1.2 and p 0.11). Nevertheless, of not existing significant difference 7 days after admission, we found that mean values for PCWP were higher in mechanically ventilated patients (mean PCWP: 10.15 versus 8.91; Student T-test: t=1.14 and p 0.12). Fourteen days after admission we have measured significantly higher values for PCWP in mechanically ventilated patients when compared to spontaneously breathing patients (mean PCWP: 10.52 versus 7.8; Student T-test: t=1.89 and p 0.032) (Table 1 and Figure 1).

Table 1. Mean values for PCWP measured in mechanically ventilated versus spontaneously breathing patients.

	Mechanically ventilated	Spontaneously breathing
Mean PCWP after 24 hours of admission	8.09	9.12
Mean PCWP after 7 days of admission	10.15	8.91
Mean PCWP after 14 days of admission	10.52	7.8

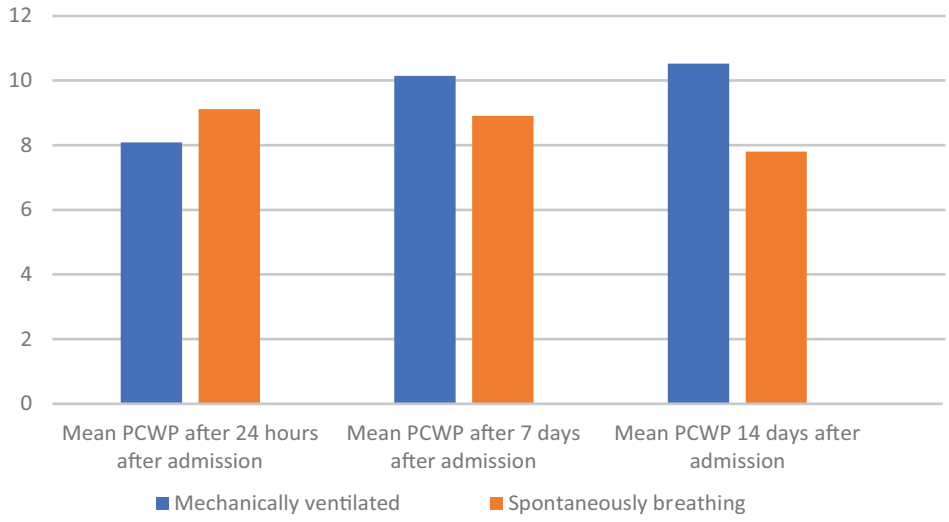

Figure 1. Mean values for PCWP measured in mechanically ventilated versus spontaneously breathing patients

Mean LUS Score after 24 hours of admission was 9.19 in mechanically ventilated patients versus 7.96 in spontaneously breathing patients without significant difference between both groups (Student T test: t=0.85 and p 0.198). Seven days after admission mean LUSS was 14.41 in mechanically ventilated patients versus 9.78 in spontaneously breathing patients. After 14 days of admission, we have found mean LUS of 13.26 in mechanically ventilated patients versus 5.72 in spontaneously breathing patients. According to above-elaborated results we have found significantly higher values of LUSS in mechanically ventilated patients 7 and 14 days after admission when compared to spontaneously breathing patients (Student T test: t=2.56 and p 0.0069 for measurements done after 7 days of admission; t=3.77 and p 0.00029 for measurements taken 14 days after admission) (Table 2).

Table 2. Mean values of LUSS in mechanically ventilated and spontaneously breathing patients and their comparison with Student T Test values.

	Mechanically ventilated	Spontaneously breathing	Student T Test: T value	Student T Test: p values
LUSS 24 hours after admission	9.19	7.96	0.85	0.198
LUSS 7 days after admission	14.41	9.78	2.56	0.0069
LUSS 14 days after admission	13.26	5.72	3.77	0.00029

The correlation between LUSS and measured PCWP in both groups 24 hours after examination, 7 and 14 days after admission was examined using Pearson Correlation Coefficient. The existence of positive correlation between measured LUSS and PCWP at every examination point in both groups was found. Values for correlation coefficient R in mechanically ventilated patients were 0.4; 0.17 and 0.46 for measurements made 24 hours after admission, 7 and 14 days after admission respectively. In spontaneously breathing patients values for R were 0.46; 0.373 and 0.269 respectively.

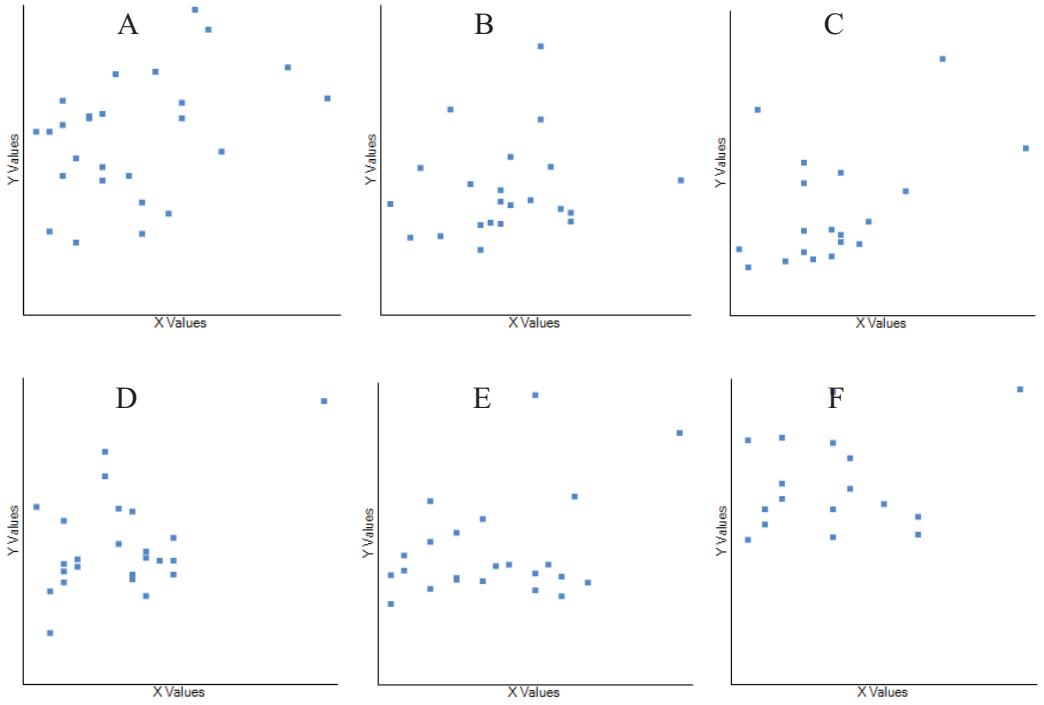

Figure 2. Correlation between LUSS and PCWP in mechanically ventilated patients 24 hours after admission (2A), 7 days after admission (2B) and 14 days after admission (2C) and in spontaneously breathing patients 24 hours after admission (2D), 7 days after admission (2E) and 14 days after admission (2F) (X values stand for LUSS; Y values stand for PCWP).

Discussion

Pulmonary Capillary Wedge Pressure (PCWP) has been recognized as a key factor in diseases that involve both heart and lungs. Since positive pressure ventilation adds pressure over the alveolar capillaries, it is expected to influence pulmonary circulation by exhibiting changes in pulmonary pressures including PCWP. Therefore, Souza R. et al. have examined the level of PCWP in patients with idiopathic pulmonary hypertension and compared it to patients experiencing ARDS concluding that ARDS has been associated with mild pulmonary hypertension (7). These findings justify the examination of PCWP in patients with pulmonary contusions, since most of them experience some sort of acute lung injury. Since elevated levels of PCWP were recognized in mechanically ventilated patients with respiratory insufficiency and had been clearly stated back in 1977, we believe that mechanical ventilation could be another source of pulmonary injury despite the primary pulmonary damage made by the kinetic energy leading to a higher level of PCWP in mechanically ventilated patients (8). According to our results it was found that levels of PCWP were higher in mechanically ventilated patients after 7 days of mechanical ventilation but were significantly higher in mechanically ventilated patients after 14 days of mechanical ventilation, initiation which implies that longer lasting positive pressure ventilation is related to higher values of PCWP. In one randomized controlled trial where systolic, diastolic pulmonary artery pressure and PCWP were examined in patients with ARDS induced by pulmonary contusions versus ARDS from other origin, it was found that the patients experiencing ARDS caused by pulmonary contusions had significantly higher values for systolic and diastolic pulmonary artery pressure as well as PCWP (9). They found that PCWP is higher 72 hours after pulmonary contusion occurrence when compared to measurements at 0 and 48 hours after admission,

which is in concordance with our findings that PCWP becomes higher as time passes after mechanical ventilation has been initiated. When compared to our study, the study of Yang W. et al. had examined the patients using Swan Ganz catheter and pulse index continuous cardiac output (PiCCO) monitoring while we were using non-invasive echocardiographic monitoring. Their study has provided results in timeframe of 0 to 72 hours, while ours have examined PCWP in an extended period of time up to 14 days of ICU admission. Compared to our study they have not specified if the patients were mechanically ventilated or not, but have confirmed that pulmonary contusions are associated with higher values for pulmonary circulation pressures (8). Moreover, they have found that higher values for PCWP were associated with elevated values of extravascular lung water which could also be estimated when using LUS Lichtenstein's BLUE Protocol as it was used in our study (9). Elevation of the amount of extravascular lung water has been related to increased microvascular permeability and due to high hydrostatic pressures (10). Regarding the relationship of the values for PCWP and the LUSS which are showing the severity of lung damage, we have found positive correlation of the measured PCWP with LUSS at 24 hours, 7 days and 14 days after admission in the ICU, which once again confirms the above-stated findings of Yang W. et al. Increased pulmonary capillary wedge pressure was met in the study of Hakim TS. et al. as well, where increasement of PEEP above 5 was associated with significant increase of PCWP elevation which confirms that positive pressure mechanical ventilation is associated with higher values for PCWP which was observed in our study when comparing to spontaneously breathing patients (11). Another study published by Slim AM and coworkers have found strong association of increased levels of PCWP in mechanically ventilated patients with positive pressure ventilation which is believed to happen not because of change in volume but rather because of elevation of intrapulmonary pressures (12). Application of continuous positive pressure ventilation in the form of nasal CPAP in patients with obstructive sleep apnea was proven to be followed by significant elevations of pulmonary pressures including PCWP which was correlated with the amount of cm of H20 of CPAP applied over the lung (13). Positive pressure ventilation in contrast to spontaneous breathing implies application of PEEP which has been already verified to be associated with higher levels of PCWP which explains our results of detecting higher levels of PCWP in those patients who were mechanically ventilated (14). Since the mean value for LUSS in both groups 24 hours after admission did not differ significantly, we believe that the severity of pulmonary contusions was similar in both examined groups. This statement implies that the severity of the initial trauma could not be the reason why mechanically ventilated patients exhibit higher PCWP values, but rather mechanical ventilation by itself leads to elevation in PCWP in patients exposed to positive pressure ventilation. The absence of invasive measurement of PCWP could be considered as a limitation in this study since that way more precise measurements could be provided and compared to noninvasively derived measurements provided by echocardiography. Invasive monitoring of PCWP is a gold standard in complex cases, but it has been related to complications with measurement, therefore a noninvasive way of monitoring pulmonary pressures using echocardiography could be a safer source of valuable data on a daily basis in critically ill patients. Further examination of pulmonary pressures on a bigger cohort of patients may be needed in order to provide deeper information about the heart lung interactions in polytraumatized patients with pulmonary contusions.

Conclusions

Using noninvasive echocardiography derived measurements, we have found higher values for PCWP in mechanically ventilated patients with pulmonary contusions when compared to spon-

taneously breathing patients. According to our findings, the duration of mechanical ventilation has been associated to significantly higher PCWP values. Since we did not find any significant difference in the severity of the initial injury, we believe that initial lung injury is not related to difference in PCWP that we have found between the groups. PCWP has correlated with severity of lung injury and the amount of extravascular lung water both represented by LUSS.

References:

1. Hosseini M, Ghelichkhani P, Baikpour M, et al. Diagnostic accuracy of ultrasonography and radiography in detection of pulmonary contusion; A systematic review and meta-analysis. Emerg (Tehran) 2015;3:127–36.
2. Kurt A, Turut H, Acipayam A, Kirbas A, Yuce S, Cumhur Cure M, Cure E. Investigation of surfactant protein-D and interleukin-6 levels in patients with blunt chest trauma with multiple rib fractures and pulmonary contusions: a cross-sectional study in Black Sea Region of Turkey. BMJ Open. 2016 Oct 12;6(10):e011797. doi: 10.1136/bmjopen-2016-011797. PMID: 27733410; PMCID: PMC5073616.
3. Nagueh SF, Middleton KJ, Kopelen HA, Zoghbi WA, Quiñones MA. Doppler tissue imaging: a noninvasive technique for evaluation of left ventricular relaxation and estimation of filling pressures. J Am Coll Cardiol. 1997 Nov 15;30(6):1527-33. doi: 10.1016/s0735-1097(97)00344-6. PMID: 9362412.
4. Bouhemad B, Nicolas-Robin A, Benois A, Lemaire S, Goarin JP, Rouby JJ. Echocardiographic Doppler assessment of pulmonary capillary wedge pressure in surgical patients with postoperative circulatory shock and acute lung injury. Anesthesiology. 2003 May;98(5):1091-100. doi: 10.1097/00000542-200305000-00011. PMID: 12717130.
5. Roberto M. Lang, MD, FASE, FESC, Luigi P. Badano, MD, PhD, FESC, Victor Mor-Avi, PhD, FASE, Jonathan Afilalo, MD, MSc, Anderson Armstrong, MD, MSc, Laura Ernande, MD, PhD, Frank A. Flachskampf, MD, FESC, Elyse Foster, MD, FASE, Steven A. Goldstein, MD, Tatiana Kuznetsova, MD, PhD, Patrizio Lancellotti, MD, PhD, FESC, Denisa Muraru, MD, PhD, Michael H. Picard, MD, FASE, Ernst R. Rietzschel, MD, PhD, Lawrence Rudski, MD, FASE, Kirk T. Spencer, MD, FASE, Wendy Tsang, MD, Jens-Uwe Voigt, MD, PhD, FESC, Recommendations for Cardiac Chamber Quantification by Echocardiography in Adults: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging, European Heart Journal - Cardiovascular Imaging, Volume 16, Issue 3, March 2015, Pages 233–271, https://doi.org/10.1093/ehjci/jev014.
6. Mojoli F, Bouhemad B, Mongodi S, Lichtenstein D. Lung ultrasound for critically ill patients. Am J Respir Crit Care Med.2019;199(6):701–714. doi: 10.1164/rccm.201802-0236CI.
7. Souza R, Amato MB, Demarzo SE, Deheinzelin D, Barbas CS, Schettino GP, Carvalho CR. Pulmonary capillary pressure in pulmonary hypertension. Crit Care. 2005 Apr;9(2):R132-8. doi: 10.1186/cc3038. Epub 2005 Feb 11. PMID: 15774045; PMCID: PMC1175923.
8. Labrousse J, Tenaillon A, Massabie P, Simonneau G, Lissac J. Influence de la ventilation artificielle sur la pression capillaire pulmonaire dans les insuffisances respiratoires aiguës (Effect of artificial ventilation on pulmonary capillary pressure in acute respiratory insufficiency). Nouv Presse Med. 1977 May 7;6(19):1639-42. French. PMID: 866151.

9. Yang WJ, Zhao XF, Wei K, Feng QG, Wang W, Liu XZ. (Clinical study on pulmonary circulation changes in patients with acute respiratory distress syndrome induced by lung contusion). Zhongguo Wei Zhong Bing Ji Jiu Yi Xue. 2012 Jul;24(7):407-11. Chinese. PMID: 22748456.

10. Benzing A, Geiger K. Inhaled nitric oxide lowers pulmonary capillary pressure and changes longitudinal distribution of pulmonary vascular resistance in patients with acute lung injury. Acta Anaesthesiol Scand. 1994 Oct;38(7):640-5. doi: 10.1111/j.1399-6576.1994.tb03970.x. PMID: 7839770.

11. Hakim TS, Gilbert E, Camporesi E. Positive end-expiratory pressure increases capillary pressure relative to wedge pressure in the closed and open chest. J Crit Care. 1993 Jun;8(2):80-6. doi: 10.1016/0883-9441(93)90011-9. PMID: 8343866.

12. Slim AM, Martinho S, Slim J, Davenport E, Castillo-Rojas LM, Shry EA. The effect of airway pressure release ventilation on pulmonary catheter readings: specifically pulmonary capillary wedge pressure in a Swine model. Anesthesiol Res Pract. 2011;2011:371594. doi: 10.1155/2011/371594. Epub 2011 Feb 24. PMID: 21403912; PMCID: PMC3049321.

13. Akashiba T, Minemura H, Yamamoto H, Itoh D, Kosaka N, Saitoh O, Horie T. Effects of nasal continuous positive airway pressure on pulmonary haemodynamics and tissue oxygenation in patients with obstructive sleep apnoea. Respirology. 1999 Mar;4(1):83-7. doi: 10.1046/j.1440-1843.1999.00159.x. PMID: 10339735.

14. Smiseth OA, Thompson CR, Ling H, Robinson M, Miyagishima RT. A potential clinical method for calculating transmural left ventricular filling pressure during positive end-expiratory pressure ventilation: an intraoperative study in humans. J Am Coll Cardiol. 1996 Jan;27(1):155-60. doi: 10.1016/0735-1097(95)00420-3. PMID: 8522690.

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TELOMERASE GENE EXPRESSION IN URINARY BLADDER CARCINOMA

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Abstract

Urinary bladder carcinoma (UBC) is the most prevalent malignancy of the urinary tract and is associated with high recurrence and mortality rates. Histologically, urothelial carcinoma is the predominant form, accounting for nearly 90% of all bladder cancer cases. One of the most frequent molecular alterations in malignant neoplasms, including UBC, is the abnormal activation of the telomerase gene.

This study aimed to quantitatively assess the expression of the TERT gene using Real-Time PCR in tissue samples obtained from patients with UBC. In total 34 patients, with histopathological confirmed urothelial bladder carcinoma, were included, along with a control group of 17 individuals without bladder malignancy.

The mean TERT gene expression level in the low-grade subgroup was 1.268 ± 0.472, compared to 2.137 ± 0.942 in the high-grade subgroup. This difference was statistically highly significant ($p< 0.0001$). Logistic regression analysis revealed that patients with high-grade UBC were 7.39 times more likely to exhibit elevated TERT gene expression compared to those with low-grade UBC ($p< 0.001$). The area under the ROC curve (AUC) was calculated to be 7.89, indicating that the model is highly effective in predicting tumor grade based on TERT expression levels.

The study established a positive correlation between histological grade and TERT gene expression levels. These findings suggest that molecular detection of abnormal telomerase transcriptional activity could serve as a useful auxiliary biomarker in the personalized management of patients with bladder carcinoma.

***Key Words**: gene expression, telomerase, TERT, urinary bladder cancer.*

Introduction

Urinary bladder carcinoma (UBC) is the most common malignancy of the urinary system and is characterized by high recurrence and mortality rates. Histologically, urothelial carcinoma is the most prevalent type, accounting for approximately 90% of all bladder cancer cases.

Urinary bladder carcinoma accounts for approximately 3% of newly diagnosed cancers globally, with men affected up to four times more than women (1, 2), The highest incidence is reported in Southeastern Europe (26.6/100,000 men), and rates are expected to rise due to aging populations and increased tobacco use (3).

According to the Cancer Registry of the Institute of Public Health of North Macedonia, there were 239 newly diagnosed cases of UBC, yielding an incidence rate of 11.52 per 100,000 inhabitants. In 2020, 137 deaths were attributed to bladder cancer (101 men and 36 women) (4).

A major challenge in managing bladder tumors is their tendency to recur and progress to higher stages and grades. Furthermore, treatment response is highly variable, as UBCs display diverse phenotypes with differing responses to surgery, chemotherapy, radiotherapy, immunotherapy and other treatments. Modern urological science within the medical field seeks to bridge the gap between tumor phenotype and genotype to better guide clinical decision-making.

To that end, there is a trend toward centralizing and standardizing preoperative diagnostic procedures for all patients with UBC. After initial transurethral resection (TUR), a treatment strategy is developed based on both the phenotypic and genotypic characteristics of the tumor.

There is a strong interest, both scientifically and clinically, in identifying molecular and genetic markers that correlate with clinical and histopathological features of UBC. These biomarkers offer potential for use in differential diagnosis, disease prognosis, therapy selection and patient monitoring.

Telomeres are repetitive DNA-protein structures at the ends of eukaryotic chromosomes that protect them from degradation, fusion and genomic instability (5, 6). Due to the end-replication problem, telomeres shorten with each cell division, eventually leading to cellular senescence or apoptosis in normal somatic cells (7). Telomerase, a specialized enzyme composed of an RNA template (coded by hTR or hTERC gene) and a catalytic subunit telomerase reverse transcriptase (coded by gene TERT), counteracts telomere shortening by adding telomeric repeats to chromosome ends (8, 9). While telomerase is active in germline, stem and some immune cells, it is typically inactive in most of the somatic cells.

In cancer, telomerase is abnormally reactivated, allowing cells to bypass senescence and achieve unlimited replicative potential- a hallmark of malignant transformation (10, 11). The TERT gene plays a central role in this process, and its expression is regulated by transcriptional and epigenetic mechanisms, including promoter binding sites and GC-rich regions that influence chromatin remodeling and methylation (12). Telomerase activation occurs in over 80% of human cancers through various telomere maintenance mechanisms, such as TERT gene mutations, rearrangements, amplifications, alternative splicing and transcription factor binding alterations, which are often tumor-type and tissue-specific (13, 14).

Our study aims to quantitatively assess TERT gene expression in patients with urothelial bladder carcinoma and examine its correlation with key clinical and pathological parameters. The ulti-

mate goal is to evaluate the potential of abnormal telomerase activity as a supportive molecular marker in guiding personalized treatment strategies for bladder cancer patients.

Materials and Methods

This study involved the quantitative determination of TERT gene expression using Real-Time PCR in tissue samples obtained from patients diagnosed with urothelial bladder carcinoma (UBC). The investigation included 34 patients with histopathological confirmed UBC and a control group of 17 patients without malignant bladder disease.

Inclusion criteria in the study required primary tumors with a histopathological confirmed diagnosis of urothelial bladder carcinoma and written informed consent from the patient. Exclusion criteria included absence of confirmed urothelial carcinoma, presence of a different tumor type, incomplete or missing key clinical data, insufficient quality of isolated DNA/RNA, age under 18 years or refusal to provide informed consent.

For each patient, selected demographic data (age and gender) and histological grade of tumor differentiation were collected. The study was approved by the Research Ethics Committee at the Doctoral School of the Faculty of Medicine in Skopje.

Tissue samples were obtained either during transurethral resection (TUR). For control purposes, samples of healthy bladder mucosa were collected from patients undergoing surgery for non-malignant pathologies with no history of bladder malignancy.

Each sample (200–300mg in weight) was preserved and transported in RNA stabilization solution (RNA Later) and stored at −80°C until analysis. Total RNA was isolated from tumor tissues using an automated nucleic acid extractor and a dedicated RNA extraction kit (Genolution).

RNA concentrations were measured using a Qubit 3.0 Fluorometer.

Quantitative TERT gene expression levels were assessed using a two-steps process involving reverse transcription and real-time PCR (qRT-PCR) amplification with fluorescent TaqMan probes, performed on the OneStep Real-Time PCR System (Applied Biosystems).

Total RNA was extracted from frozen TUR-obtained tissue samples using TRI-reagent. Complementary DNA (cDNA) synthesis was performed via reverse transcription using High-Capacity cDNA Reverse Transcription Kits (Thermo-Fisher), following the manufacturer's instructions.

The following oligonucleotide primers and TaqMan probes were used:

- TERT gene:
  - Forward primer (A): 5'-GCA TTG GAA TCA GAC AGC AC-3',
  - Reverse primer (R): 5'-CCA CGA CGT AGT CCA TGT TC-3',
  - TaqMan probe: 5'FAM-CGC CCT GCT GAC GTC CAG AC-NFQ-3'.

- Reference gene GAPDH:
  - Forward primer (A): 5'-ATG GGT GTG AAC CAT GAG AA-3',
  - Reverse primer (R): 5'-GTG CTA AGC AGT TGG TGG TG-3',
  - TaqMan probe: 5'FAM-CCT CAA GAT CAT CAG CAA TGC CTC C-NFQ-3'.

The amplification protocol included:

- Polymerase activation: 10 minutes,
- 40 cycles of:
  - Denaturation at 95°C for 15 seconds,
  - Combined annealing and elongation at 70°C for 1 minute.

A negative control (ddH_2O) was used for each master mix to validate the assay.

TERT gene expression levels were calculated using the Livak method (2^−ΔCt), relative to the expression of the housekeeping gene GAPDH. The ΔCt value was determined by subtracting the Ct (threshold cycle) value of GAPDH from the Ct of TERT for each sample.

The differential expression of TERT was presented as the relative fold-change compared to GAPDH, expressed as $\log_{10}(RQ)$ for consistency across samples.

Statistical methods were applied to determine correlations between clinical/ histopathological data and molecular genetic findings. Descriptive statistics were used to analyze and present relevant demographic and clinical characteristics of the patients.

Correlation between the clinical and histopathological parameters and the molecular genetic results, was assessed using logistic regression analysis. Parametric values with normal distribution were evaluated using a two-tailed Student's t-test, whereas deviations from normal distribution were analyzed using the non-parametric Mann-Whitney U-test.

The suitability of TERT gene expression levels for logistic regression analysis was verified using the Hosmer–Lemeshow goodness-of-fit test. The odds ratio (OR) and 95% confidence interval (CI) were calculated, with a significance threshold of $p< 0.05$.

All statistical analyses were performed using XLSTAT 2016 and Microsoft Excel 2016 software.

This study presents data from 34 patients with histologically confirmed urothelial bladder carcinoma (UBC), along with 17 control samples from individuals without malignant bladder disease.

Tumor Grade Distribution

Based on histological evaluation, 11 patients were classified as having low-grade tumors and 23 as having high-grade tumors (Table 1, Figure 1).

Table 1. Histological Grade Distribution among UBC Patients.

Grade	n	%
Low Grade	11	32.35%
High Grade	23	67.65%
Total	34	100.00%

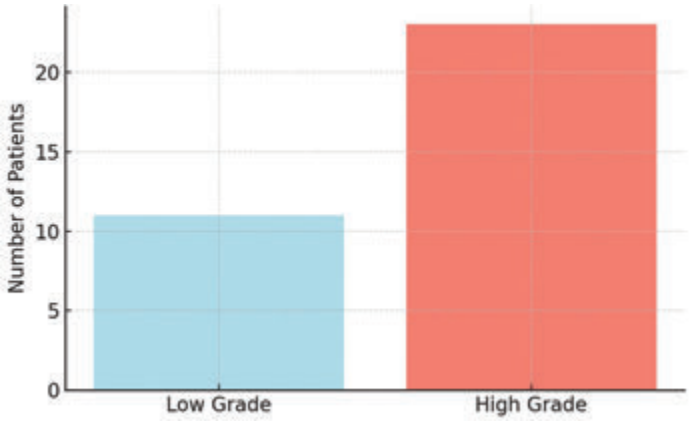

Figure 1. Distribution of Histological Grades in UBC Patients.

Gender Distribution

Gender distribution in both histological subgroups is shown in Table 2 and Figure 2.

Table 2. Gender Distribution by Tumor Grade.

Gender	Low Grade (n/%)	High Grade (n/%)	Fisher's Exact Test (p)
Male	9 (81.82%)	18 (78.26%)	1.000
Female	2 (18.18%)	5 (21.74%)	
Total	11 (100.00%)	23 (100.00%)	

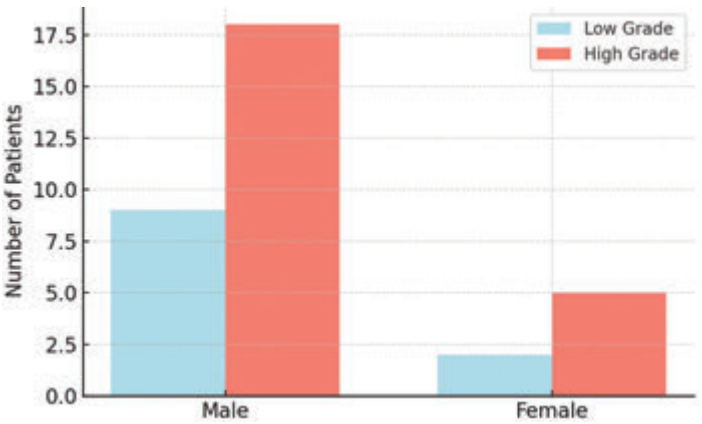

Figure 2. Gender Distribution Across Tumor Grades.

Age Distribution

The age distribution of the two subgroups is shown in Table 3.

Table 3. Age Structure by Tumor Grade.

Parameter (years)	Low Grade	High Grade	Student's t-test (p)
n	11	23	0.745
Mean Age	66.18	63.04	
SD	10.57	8.14	
Min Age	47	46	
Max Age	80	76	

There was no statistically significant difference in age or gender distribution between the two tumor grade groups ($p > 0.05$), supporting their comparability for further analysis.

TERT Gene Expression Levels

TERT gene expression levels, normalized to the GAPDH reference gene, were evaluated across the two histological subgroups. The results are presented in Table 4 and Figure 3.

Table 4. TERT Gene Expression by Tumor Grade.

hTERT Expression	Low Grade	High Grade	Mann-Whitney Test (p)
n	11	23	< 0.001
Mean	1.268	2.137	
SD	0.472	0.942	
Min	0.422	0.671	
Max	2.156	4.996	

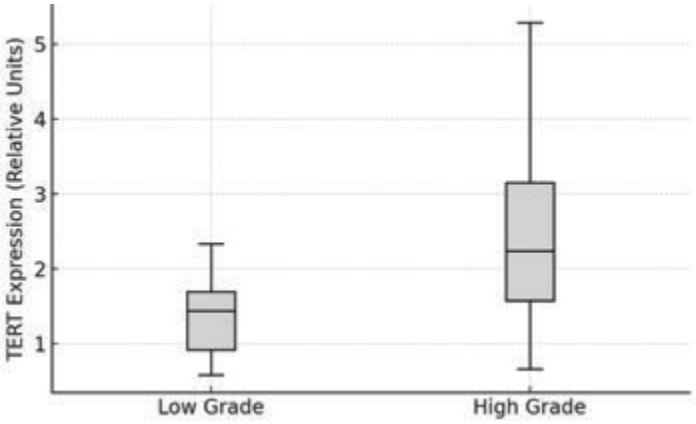

Figure 3. TERT Expression in Low vs. High-Grade UBC.

The data clearly demonstrate significantly higher TERT gene expression levels in high-grade tumors compared to low-grade ones. The mean expression level in the high-grade subgroup was

2.137 ± 0.942, while in the low-grade subgroup, it was 1.268 ± 0.472. This difference is statistically highly significant (p< 0.001), confirming a strong and proportional correlation between TERT gene expression and histological grade in urothelial bladder carcinoma.

Logistic Regression Analysis

Logistic regression analysis confirmed that increased TERT expression is significantly associated with higher tumor grades. Patients with high-grade tumors were 7.39 times more likely to exhibit elevated TERT expression (p= 0.001).

Table 5. Logistic Regression – TERT Expression and Tumor Grade.

Parameter	β Coefficient	SE	Wald χ^2	p-value	OR (95% CI)
Grade	1.073	0.318	11.36	0.001	7.39 (2.07 – 25.85)

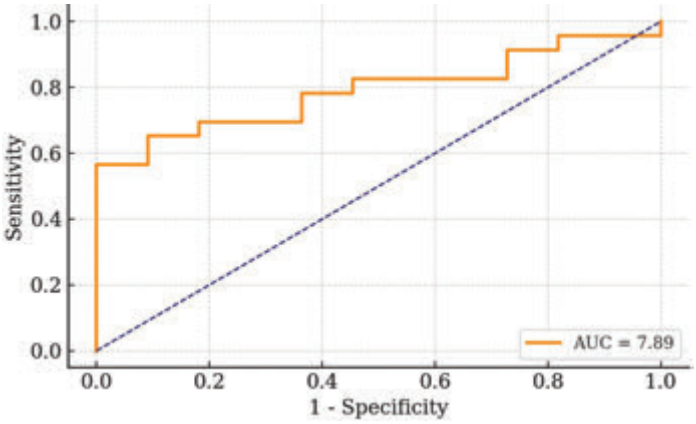

Figure 4. ROC Curve of the Predictive Model.

The area under the ROC curve (AUC) of 7.89 indicates excellent model accuracy in distinguishing high-grade from low-grade tumors based on TERT expression.

The model's predictive performance, based on TERT expression levels, is summarized in Table 6. It correctly classified tumor grades in 76.47% of cases.

Table 6. Predictive Accuracy of TERT-Based Model.

Metric	Value
Sensitivity	91.30%
Specificity	63.64%
Overall Accuracy	76.47%

Discussion

Telomerase activity is abnormally elevated in over 80% of malignant tumors and is considered to be a key factor in cellular immortalization and tumor progression (15). Numerous studies have confirmed the reactivation of telomerase in various cancers, including urothelial bladder car-

cinoma (UBC), where its activity may contribute to the malignant transformation of urothelial cells (16).

In this study, the transcriptional activity of telomerase was assessed through quantitative measurement of TERT gene expression levels in tumor tissue from 34 patients with UBC. The results were normalized against GAPDH and compared to 17 control samples from healthy bladder mucosa. A statistically significant increase in TERT expression was observed in tumor tissues compared to controls, which aligns with previously published studies on bladder cancer (17, 18, 19, 20, 21).

Notably, TERT expression levels were significantly higher in high-grade tumors compared to low-grade ones (mean values: 2.137 vs. 1.268, $p< 0.001$), suggesting a correlation between telomerase activity and tumor aggressiveness. These findings are consistent with earlier reports that linked elevated telomerase expression with higher histological grade and advanced pathological stage (22, 23).

Furthermore, logistic regression analysis showed that patients with high-grade tumors were 7.39 times more likely to exhibit increased TERT expression than those with low-grade tumors, with excellent predictive value supported by an AUC of 7.89. The model demonstrated a sensitivity of 91.30% and correctly classified tumor grade in over 76% of the cases.

Previous studies also suggest that TERT overexpression is associated with poor prognosis in various tumor types, including colorectal cancer, supporting its broader relevance as a prognostic marker (24).

Collectively, the current findings reinforce the potential clinical value of TERT gene expression in stratifying bladder cancer patients and guiding treatment strategies based on tumor biology.

Conclusion

This study confirmed a positive correlation between the histological grade of urothelial bladder carcinoma and the quantitative expression levels of the TERT gene. The patients with high-grade tumors exhibited significantly higher TERT expression than those with low-grade tumors.

The results suggest that molecular detection of abnormal telomerase transcriptional activity may serve as a supportive molecular biomarker in the personalized management of bladder cancer. Its use could aid in risk stratification, prognosis, and therapeutic decision-making, particularly in settings where tumor aggressiveness needs to be evaluated beyond standard histopathology.

References:

1. IARC, Cancer Today. Estimated number of new cases in 2020, worldwide, both sexes, all ages. 2021. [Access date: March 2022] https://gco.iarc.fr/today/online-analysis-table.
2. Sung H, Ferlay J, Siegel RL, Laversanne M et al. Global Cancer Statistics 2020,
3. GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 May;71(3):209-249. doi: 10.3322/caac.21660. Epub 2021 Feb 4. PMID: 33538338.

4. Регистар за рак во Република Северна Македонија. Институт за јавно здравје, Скопје, 2021.
5. Blackburn EH. The end of the (DNA) line. Nat Struct Biol. 2000 Oct;7(10):847-50. doi: 10.1038/79594.
6. De Lange T. How telomeres solve the end-protection problem. Science. 2009 Nov 13;326(5955):948-52. doi: 10.1126/science.1170633.
7. Huffman KE, Levene SD, Tesmer VM et al. Telomere shortening is proportional to the size of the G-rich telomeric 3'-overhang. J Biol Chem. 2000 Jun 30;275(26):19719-22. doi: 10.1074/jbc.M002843200.
8. Greider CW. Molecular biology. Wnt regulates TERT--putting the horse before the cart. Science. 2012 Jun 22;336(6088):1519-20. doi: 10.1126/science.1223785.
9. Dwyer J, Li H, Xu D, Liu JP. Transcriptional regulation of telomerase activity: roles of the the Ets transcription factor family. Ann N Y Acad Sci. 2007 Oct;1114:36-47. doi: 10.1196/annals.1396.022.
10. Daniel M, Peek GW, Tollefsbol TO. Regulation of the human catalytic subunit of telomerase (hTERT). Gene. 2012 May 1;498(2):135-46. doi: 10.1016/j.gene.2012.01.095. Epub 2012 Feb 13.
11. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011 Mar 4;144(5):646-74. doi: 10.1016/j.cell.2011.02.013.
12. Kyo S, Takakura M, Fujiwara T, Inoue M. Understanding and exploiting hTERT promoter regulation for diagnosis and treatment of human cancers. Cancer Sci. 2008 Aug;99(8):1528-38. doi: 10.1111/j.1349-7006.2008.00878.x.
13. Gaspar TB, Sá A, Lopes JM et al. Telomere Maintenance Mechanisms in Cancer. Genes (Basel). 2018 May 3;9(5):241. doi: 10.3390/genes9050241.
14. Dratwa M, Wysoczańska B, Łacina et al. TERT-Regulation and Roles in Cancer Formation. Front Immunol. 2020 Nov 19;11:589929. doi: 10.3389/fimmu.2020.589929.
15. Shay JW, Wright WE. Telomeres and telomerase: three decades of progress. Nat Rev Genet. 2019 May;20(5):299-309. doi: 10.1038/s41576-019-0099-1.
16. Tsatsakis A, Oikonomopoulou T, Nikolouzakis TK et al. Role of telomere length in human carcinogenesis (Review). Int J Oncol. 2023 Jul;63(1):78. doi: 10.3892/ijo.2023.5526. Epub 2023 May 26.
17. Okumura A, Mizuno I, Nagakawa O et al. Telomerase activity is correlated with lower grade and lower stage bladder carcinomas. Int J Urol. 2004 Dec;11(12):1082-6. doi: 10.1111/j.1442-2042.2004.00960.x.
18. Jafri MA, Ansari SA, Alqahtani MH et al. Roles of telomeres and telomerase in cancer, and advances in telomerase-targeted therapies. Genome Med. 2016 Jun 20;8(1):69. doi: 10.1186/s13073-016-0324-x.
19. Bravaccini S, Sanchini MA, Granato AM et al. Urine telomerase activity for the detection of bladder cancer in females. J Urol. 2007 Jul;178(1):57-61. doi: 10.1016/j.juro.2007.03.025. Epub 2007 May 11.
20. Morii A, Komiya A, Okumura A et al. Telomerase activity in bladder cancer tissue. Exp Ther Med. 2010 Jan;1(1):85-88. doi: 10.3892/etm_00000015. Epub 2010 Jan 1.

21. Zhang B, Bai YX, Ma HH et al. Silencing PinX1 compromises telomere length maintenance as well as tumorigenicity in telomerase-positive human cancer cells. Cancer Res. 2009 Jan 1;69(1):75-83. doi: 10.1158/0008-5472.CAN-08-1393.

22. Lin Y, Miyamoto H, Fujinami K et al. Telomerase activity in human bladder cancer. Clin Cancer Res. 1996 Jun;2(6):929-32.

23. Takihana Y, Tsuchida T, Fukasawa M et al. Real-time quantitative analysis for human telomerase reverse transcriptase mRNA and human telomerase RNA component mRNA expressions as markers for clinicopathologic parameters in urinary bladder cancer. Int J Urol. 2006 Apr;13(4):401-8. doi: 10.1111/j.1442-2042.2006.01300.x .

24. Tatsumoto N, Hiyama E, Murakami Y et al. High telomerase activity is an independent prognostic indicator of poor outcome in colorectal cancer. Clin Cancer Res. 2000 Jul;6(7):2696-701.

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CORRELATION OF GLUCOSE AND LIPID STATUS IN PATIENTS WITH GESTATIONAL DIABETES MELLITUS

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Abstract

Introduction: Gestational diabetes mellitus, characterized by glucose intolerance during pregnancy, poses heightened risks to both the mother and fetus. It emerges due to increased insulin resistance during pregnancy. Adipose tissue plays a vital role in regulating various biological processes through the secretion of adipokines, which influence both pregnancy and the development of gestational diabetes. Hyperlipidemia, a well-known contributor to atherosclerosis, directly impacts the onset of cardiovascular diseases. Pregnancy leads to an increase in serum levels of total cholesterol and triglycerides, driven by increased levels of hormones such as estrogens, progesterone and lactogen.

Material and Methods: This study presents prospective clinical research involving 65 individuals, excluding 6 patients with spontaneous abortion. A total of 59 patients were incorporated into the statistical analysis. The patients underwent three follow-up visits. During the initial visit, the participants were enrolled in the study; all of them were healthy individuals in the first trimester of pregnancy. In the second visit, an oral glucose tolerance test (OGTT) with 75 grams of glucose was conducted between 24 and 28 weeks of gestation, and the patients were categorized. Patients exhibiting a positive OGTT were diagnosed with gestational diabetes. The third visit occurred in the third trimester of gestation.

Results: Gestational diabetes mellitus was registered in 14 (23.73%) patients. Body mass index had significantly higher values in the group with gestational diabetes (34.59 ± 3.9 vs 29.95 ± 5.4 kg/m2, p=0.0044). The comparison of the two groups regarding the lipid status presented significantly higher triglycerides in the group with gestational diabetes (4.01 ± 2.3 vs 2.62 ± 0.9, (p=0.0017). The other parameters of lipid status were similar between the two groups. In both groups, the changes in glucose parameters were statistically insignificant in the third, compared to the first trimester of pregnancy.

Conclusion: Dyslipidemia during pregnancy is a common but complex condition with consequences for both the mother and the fetus.

Key Words: *dyslipidemia;gestational diabetes; glycemia; triglycerides.*

Introduction

Gestational diabetes mellitus (GDM) is a form of glucose intolerance that develops during pregnancy, with a global prevalence of 17.8% (1). It results from increased insulin resistance driven by hormonal changes and adipokine activity from maternal adipose tissue (1). Women with GDM face a two- to threefold increased risk of developing type 2 diabetes later in life, while their offspring are predisposed to obesity and metabolic disorders. Risk factors include obesity, advanced maternal age, family history of diabetes, polycystic ovarian syndrome, hypertensive disorders and prior poor pregnancy outcomes (2). Obesity-associated inflammation and placental cytokines play a key role in disease onset. GDM is linked to maternal complications such as preeclampsia, gestational hypertension and cesarean delivery, and fetal risks including macrosomia, shoulder dystocia, congenital anomalies, neonatal hypoglycemia and increased NICU admissions (2,3).

Physiological changes in lipid metabolism during pregnancy lead to a progressive increase in total cholesterol, triglycerides and low-density lipoprotein cholesterol (LDL), peaking in the third trimester (4,5). While these changes support fetal development, excessive lipid levels—particularly in women with high BMI or preexisting dyslipidemia—may worsen maternal endothelial dysfunction and contribute to pregnancy complications such as preeclampsia (6–8). Despite their potential impact, lipid levels are not routinely monitored during pregnancy.

Understanding the interplay between glucose and lipid metabolism in pregnancy is crucial, particularly in high-risk populations.

Therefore, this study aims to determine whether there is a significant difference in lipid and glucose profiles between patients with gestational diabetes and healthy patients.

Material and Methods

This prospective clinical study was performed at the University Clinic for Endocrinology, Diabetes and Metabolic Diseases in Skopje, Republic of North Macedonia, for a 12-month duration, from March 2022 to March 2023. The main aim of the study was to evaluate metabolic alterations throughout pregnancy, specifically regarding the onset of gestational diabetes mellitus (GDM) and related biochemical indicators.

Study Cohort. The study initially recruited 65 clinically healthy pregnant women in their first trimester. The inclusion criteria were singleton pregnancies, no pre-existing diabetes or metabolic disorders, and consent to participate in all phases of the trial. Individuals with chronic conditions, numerous gestations, or those who did not attend follow-up appointments were omitted. From the initial cohort, six patients experienced spontaneous abortions during the study and were therefore eliminated from the final statistical analysis. Consequently, the ultimate study cohort consisted of 59 patients.

Research Methodology and Subsequent Monitoring. Participants were prospectively monitored during three clinical visits corresponding to the first, second, and third trimesters of pregnancy.

Initial Consultation (First Trimester): This appointment occurred during the early stage of gestation (gestational age ≤13 weeks). Comprehensive demographic and medical history were doc-

umented for each participant, encompassing age, gestational age, gravidity, parity and familial diabetes history. Baseline laboratory assessments were conducted, encompassing fasting plasma glucose (FPG), fasting insulin levels and serum lipid profile (triglycerides, total cholesterol, high-density lipoprotein [HDL] and low-density lipoprotein [LDL].

During the second visit, occurring between the 24th and 28th week of gestation, all participants underwent an oral glucose tolerance test (OGTT) utilizing a 75-gram glucose load, in line with the standards established by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) and the World Health Organization (WHO) in 2013. Patients were categorized into two groups based on the OGTT results: those diagnosed with gestational diabetes mellitus (GDM) and those exhibiting normal glucose tolerance (NGT).

- Third Visit (Third Trimester): The final clinical assessment occurred during the third trimester (gestational age ≥30 weeks). During this visit, laboratory tests were conducted again, encompassing fasting plasma glucose, fasting insulin levels and blood lipids (triglycerides, total cholesterol, HDL, LDL). The readings were compared to the baseline (first trimester) data to assess metabolic changes throughout pregnancy.

Data Acquisition and Biochemical Assessment.All biochemical assays were conducted in the clinic's central laboratory utilizing standardized enzymatic and immunoassay techniques. Blood samples were collected following a minimum overnight fast of 8 hours. Glucose concentrations were assessed with the hexokinase technique. Insulin was quantified using chemiluminescent immunoassay, whereas lipid parameters were assessed by enzymatic colorimetric methods.

Statistical Analysis

The statistical analysis of the data obtained from the study was performed using the statistical program SPSS 23.0. Shapiro Wilk's test was used to test the normality of the data distribution. The obtained data are presented in tables and graphs.

Categorical (attribute) variables are presented with absolute and relative numbers. Numerical (quantitative) variables are presented with mean, standard deviation, minimum and maximum values.

Student t-test for independent samples was used to compare the two groups.

Student t-test for dependent samples was used to test the difference in changes in the analyzed parameters in the third versus the first trimester of pregnancy.

Statistical significance was defined at the level of $p<0.05$.

Results

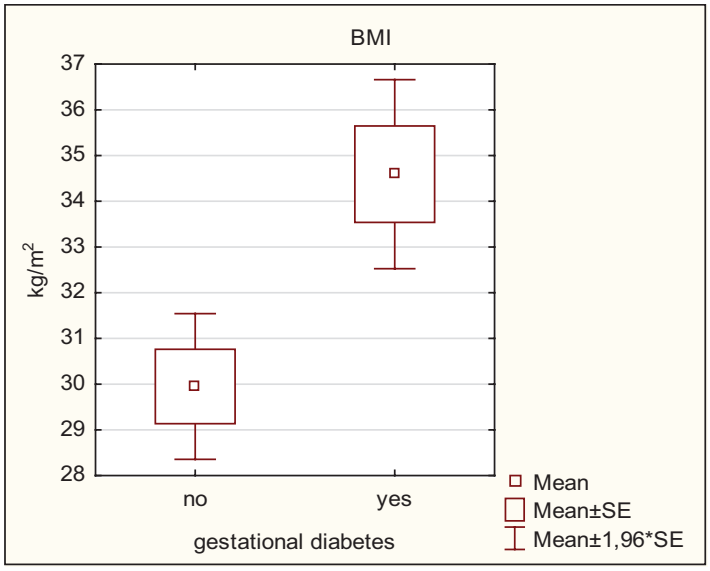
The occurrence of gestational diabetes mellitus was registered in 14 (23.73%) patients. With and without gestational diabetes were homogeneous in terms of age, i.e., patients from both groups had similar ages (31.4 ± 4.2 vs 30.0 ± 4.7 years; t=1.01 p=0.0044).

The body mass index had significantly higher values in the group with gestational diabetes (34.59 ± 3.9 vs 29.95 ± 5.4 kg/m2, p=0.0044).

Table 1. Age and body mass index of the patients with and without gestational diabetes.

Variable	Gestational Diabetes		p-level
	yes	no	
Age (years) mean ±. SD Min - max	31.4 ± 4.2 24 – 39	30.0 ± 4.7 20 – 39	t=1.01 p=0.32
BMI (kg/m$_2$) mean ± SD Min - max	34.59 ± 3.9 29.8 – 41.1	29.95 ± 5.4 22.4 – 44	t=2.96 **p=0.0044

The values are shown with mean ± SD, min-max, BMI: body mass index
T (Student t-test for independent samples),**sig p<0.01



Graph 1. Graphical presentation of average BMI in patients with/without Gestational diabetes.

Patients with and without gestational diabetes did not differ significantly in terms of glucose status: they had similar values of insulin (13.67 ± 6.9 vs 14.49 ± 9.9, p=0.77), fasting plasma glucose (4.80 ± 0.5 vs 4.54 ± 0.4, p=0.06), and HbA1c (5.22 ± 0.4 vs 5.25 ± 0.3, p=0.76).

Table 2. Insulin, glycaemia and HbA1c in patients with and without gestational diabetes.

variable	Gestational Diabetes		p-level
	yes	no	
Insulin mean ±. SD Min - max	 13.67 ± 6.9 6.22 – 27.41	 14.49 ± 9.9 3.63 – 51.88	 t=0.3 p=0.77
Glycaemia mean ±. SD Min - max	 4.80 ± 0.5 4.05 – 5.8	 4.54 ± 0.4 3.6 – 5.65	 t=1.9 p=0.06
HbA1c mean ±. SD Min - max	5.22 ± 0.4 4.32 – 6.12	5.25 ± 0.3 4.43 – 6.04	t=0.29 p=0.76

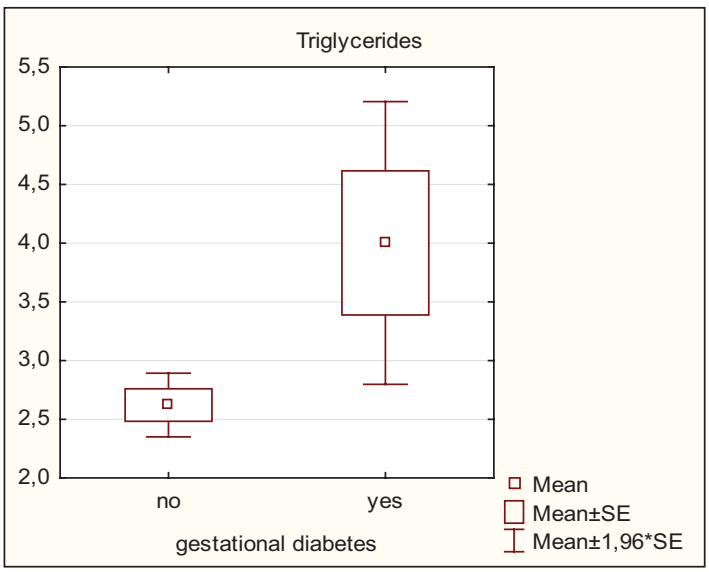
The values are shown with mean ± SD, min-max, T (Student t-test for independent samples)

Comparison of the two groups in terms of lipid status presented significantly higher triglycerides in the group with gestational diabetes (4.01 ± 2.3 vs 2.62 ± 0.9, p=0.0017). Other lipid status parameters were similar between the two groups: total cholesterol was insignificantly higher in the group with gestational diabetes (7.13 ± 1.02 vs 7.13 ± 1.02, p=0.068); HDL had insignificantly lower values in the group with gestational diabetes (1.72 ± 0.4 vs 1.77 ± 0.4, p=0.68); LDL was insignificantly higher in the group with gestational diabetes (4.04 ± 1.1 vs 3.95 ± 0.98, p=0.3).

Table 3. Comparison of the two groups in terms of lipid status.

variable	Gestational Diabetes		p-level
	yes	no	
Triglycerides mean ±. SD Min - max	4.01 ± 2.3 1.95 – 10.82	2.62 ± 0.9 0.98 – 5.61	t=3.3 **p=0.0017
Total cholesterol mean ±. SD Min - max	7.13 ± 1.02 5.67 – 9.46	6.51 ± 1.1 4.36 – 9.07	t=1.9 p=0.06
HDL mean ±. SD Min - max	1.72 ± 0.4 1.11 – 2.56	1.77 ± 0.4 0.99 – 2.7	t=0.4 p=0.68
LDL mean ±. SD Min - max	4.04 ± 1.1 1.28 – 5.65	3.95 ± 0.98 2.34 – 6.41	t=0.3 p=0.8

The values are shown with mean ± SD, min-max ,HDL: high-density lipoprotein, LDL: low-density lipoprotein. t (Student t-test for independent samples) **sig p<0.01



Graph 2. Graphical presentation of average triglycerides in patients with/without Gestational diabetes.

In both groups, changes in glucose parameters were statistically insignificant in the third trimester of pregnancy compared to the first trimester, while all lipid status parameters were significantly higher.

Greater changes in lipid status were registered in the group with gestational diabetes, except for

the LDL parameter: the average change for triglycerides was 2.397 vs 1.34, for total cholesterol 2.17 vs 1.79, for HDL 0.27 vs 0.17.

Table 4. Change in clinical parameters.

	variable	1	3	difference	p-level
GD	Insulin	14.36 ± 5.1	13.67 ± 6.9	0.698	t=0.3 p=0.77
	Glycaemia	4.84 ± 0.6	4.80 ± 0.5	0.038	t=0.2 p=0.86
	HbA1c	5.29 ± 0.3	5.22 ± 0.4	0.077	t=0.8 p=0.42
	Triglycerides	1.61 ± 0.6	4.01 ± 2.3	2.397	t=3.7 **p=0.0025
	Total cholesterol	4.96 ± 1.1	7.13 ± 1.0	2.17	t=7.0 ***p=0.000009
	HDL	1.45 ± 0.2	1.72 ± 0.4	0.27	t=2.2 *p=0.048
	LDL	2.98 ± 0.7	4.04 ± 1.1	1.05	t=3.7 **p=0.0028
	Weight	86.0 ± 10.5	92.14 ± 11.5	6.14	t=6.4 ***p=0.00002
	BMI	31.93 ± 3.7	34.59 ± 3.9	2.66	t=4.6 ***p=0.0005
Without GD	Insulin	12.96 ± 7.6	14.49 ± 9.9	1.53	t=1.3 p=0.19
	Glycaemia	4.68 ± 0.5	4.54 ± 0.4	0.139	t=1.8 p=0.08
	HbA1c	5.23 ± 0.2	5.25 ± 0.3	0.026	t=0.5 p=0.63
	Triglycerides	1.26 ± 0.8	2.62 ± 0.9	1.34	t=9.3 ***p=0.000000
	Total cholesterol	4.71 ± 0.98	6.51 ± 1.1	1.79	t=9.8 *** p=0.000000
	HDL	1.59 ± 0.4	1.77 ± 0.4	0.17	t=2.9 **p=0.005
	LDL	2.73 ± 0.8	3.95 ± 0.98	1.22	t=7.3 ***p=0.000000
	Weight	72.04 ± 15.1	81.0 ± 14.7	8.95	t=14.4 ***p=0.000000
	BMI	26.70 ± 5.4	29.95 ± 5.4	3.24	t=11.3 *** p=0.000000

The values are shown with ± SD, min-max, HDL: high-density lipoprotein, LDL: low-density lipoprotein; BMI: body mass index, t (Student t-test for dependent samples), *sig p<0.05; **sig p<0.01; ***sig p<0.0001

Discussion

This prospective study demonstrated a correlation between glucose and lipid status in patients with gestational diabetes and healthy pregnant patients. The age at conception was comparable between the two groups, reinforcing the established understanding that, in addition to maternal age, other major risk factors for GDM include family history and obesity (9). Our findings confirmed that the body mass index was significantly higher in the group of patients with gestational diabetes compared to the group of healthy subjects. The analysis of insulin, fasting plasma glucose and HbA1c in the third trimester of pregnancy between the group of patients with gestational diabetes and the group of healthy subjects did not differ significantly in terms of glucose status: they had similar values of insulin (13.67 ± 6.9 vs 14.49 ± 9.9, p=0.77), fasting plasma glucose (4.80 ± 0.5 vs 4.54 ± 0.4, p=0.06), and HbA1c (5.22 ± 0.4 vs 5.25 ± 0.3, p=0.76). Even in the group of patients with diagnosed gestational diabetes, there is a slightly lower value of HbA1c, that may reflect the effects of early diagnosis and adherence to a prescribed regimen including dietary modifications, physical activity, and pharmacological therapy, contributing to

better glycemic control.

Comparing the two groups of patients with gestational diabetes to the group of healthy participants in the third trimester of pregnancy revealed that the gestational diabetes group exhibited significantly elevated triglyceride levels. Other lipid status parameters were comparable between the two groups: total cholesterol was marginally elevated in the gestational diabetes group (7.13 ± 1.02 vs 7.13 ± 1.02, p=0.068); HDL levels were slightly reduced in the gestational diabetes group (1.72 ± 0.4 vs 1.77 ± 0.4, p=0.68); LDL was marginally higher in the gestational diabetes group (4.04 ± 1.1 vs 3.95 ± 0.98, p=0.3). The significant longitudinal increase in triglycerides and the insignificantly elevated total cholesterol and LDL in the gestational diabetes group align with the findings of Farias et al., which demonstrated a linear increase in total cholesterol, LDL and triglycerides with advancing gestational weeks (10). Only HDL has the highest value throughout the third trimester before commencing a drop.

The study by Lippi et al. showed that gestational age markedly affected lipid parameters, with women in their second and third trimesters showing significantly elevated levels of total cholesterol, low-density lipoprotein cholesterol and triglycerides (11). Likewise, Alvarez et al. observed that triglyceride and cholesterol concentrations escalated across all lipoprotein fractions as pregnancy advanced (12).

A study was conducted to analyze the correlation of glucose parameters, including insulin, fasting plasma glucose and HbA1c, between the first and third trimesters of pregnancy. The results showed no significant difference in glucose parameters in the third trimester compared to the first trimester, while all lipid status parameters were significantly higher. The lack of significant difference in glucose parameters may be attributed to the introduced treatment, dietary changes, and increased physical activity. Greater changes in lipid status were observed in the group with gestational diabetes compared to healthy subjects, except for the LDL parameter. The average change for triglycerides was 2.397 vs 1.34, for total cholesterol 2.17 vs 1.79, and for HDL 0.27 vs 0.17. Increased body weight and obesity are serious risk factors for gestational diabetes, and patients with gestational diabetes have a higher lipid profile, especially triglycerides, compared to healthy subjects. It is essential to consider the physiological increase in total cholesterol by 25-50%, LDL by 60% and triglycerides by 200%. Therefore, overall laboratory evaluation in women before pregnancy is useful to identify patients with lipid profile disorders preconceptionally, requiring intervention with diet, physical activity and medication treatment.

Despite the availability of various treatment options for dyslipidemia in pregnant patients, only bile acid sequestrates are allowed during pregnancy. Ezetimibe and fenofibrate may be considered if the benefits outweigh the potential risks. Statins are contraindicated for use, but recent meta-analyses have shown potential benefits in strictly selected cases, especially in patients with high cardiovascular risk, recent cardiac events, established cardiovascular disease, or familial hypercholesterolemia. In these cases, the final decision should carefully consider the potential risk of discontinuing therapy. More information is needed about the treatment of dyslipidemia during pregnancy with new drugs like PCSK9 inhibitors, especially Inclisiran, which can be used before pregnancy and immediately after delivery, at intervals of 9 months between treatments. The decision to treat dyslipidemia during pregnancy should be individualized.

Conclusion

Managing dyslipidemia during pregnancy necessitates a meticulous and personalized strategy, alongside a comprehensive approach, to ensure the well-being of both mother and fetus. Physiological alterations during gestation frequently intensify lipid irregularities, especially increased triglycerides and LDL-cholesterol, potentially complicating pre-existing dyslipidemic disorders. Effective care predominantly depends on lifestyle modifications and nutritional changes, with pharmacological treatment reserved for specific high-risk situations. Inherited conditions like familial hyperchylomicronemia, necessitate individualized nutritional approaches to mitigate risks and promote positive pregnancy results.

References:

1. American Diabetes Association; 14. Management of Diabetes in Pregnancy: *Standards of Medical Care in Diabetes—2021*. *Diabetes Care* 1 January 2021; 44 (Supplement_1): S200–S210.
2. Agarwal MM, Dhatt GS, Zayed R, Bali N. Gestational diabetes: relevance of diagnostic criteria and preventive strategies for Type 2 diabetes mellitus. Arch Gynecol Obstet. 2007 Sep;276(3):237-43. doi: 10.1007/s00404-007-0334-4. Epub 2007 Feb 21.
3. Agarwal MM, Dhatt GS, Zayed R, Bali N. Gestational diabetes: relevance of diagnostic criteria and preventive strategies for Type 2 diabetes mellitus. Arch Gynecol Obstet. 2007 Sep;276(3):237-43. doi: 10.1007/s00404-007-0334-4. Epub 2007 Feb 21.
4. Nasioudis D, Doulaveris G, Kanninen TT. Dyslipidemia in pregnancy and maternal-fetal outcome. Minerva Ginecol. 2019 Apr;71(2):155-162. doi: 10.23736/S0026-4784.18.04330-7. Epub 2018 Oct 11.
5. Piechota W, Staszewski A. Reference ranges of lipids and apolipoproteins in pregnancy. Eur J Obstet Gynecol Reprod Biol. 1992 Jun 16;45(1):27-35. doi: 10.1016/0028-2243(92)90190-a.
6. Herrera E, Ortega-Senovilla H. Lipid metabolism during pregnancy and its implications for fetal growth. Curr Pharm Biotechnol. 2014;15(1):24-31. doi: 10.2174/1389201015666140330192345.
7. Barbir M, Pottle A, Bornstein SR. The implications of measuring lipoprotein(a) in clinical practice. Glob Cardiol Sci Pract. 2024 Aug 1;2024(4):e202440. doi: 10.21542/gcsp.2024.40.
8. Lowe WL Jr, Karban J. Genetics, genomics and metabolomics: new insights into maternal metabolism during pregnancy. Diabet Med. 2014 Mar;31(3):254-62. doi: 10.1111/dme.12352.
9. Poornima IG, Indaram M, Ross JD, Agarwala A, Wild RA. Hyperlipidemia and risk for preclampsia. J Clin Lipidol. 2022 May-Jun;16(3):253-260. doi: 10.1016/j.jacl.2022.02.005. Epub 2022 Feb 20.
10. Lindsay KL, Brennan L, Rath A, Maguire OC, Smith T, McAuliffe FM. Gestational weight gain in obese pregnancy: impact on maternal and foetal metabolic parameters and birthweight. J Obstet Gynaecol. 2018 Jan;38(1):60-65. doi: 10.1080/01443615.2017.1328670. Epub 2017 Aug 6.
11. Lippi G, Albiero A, Montagnana M, Salvagno GL, Scevarolli S, Franchi M, Guidi GC. Lipid and lipoprotein profile in physiological pregnancy. Clin Lab. 2007;53(3-4):173-7.

12. Alvarez JJ, Montelongo A, Iglesias A, Lasunción MA, Herrera E. Longitudinal study on lipoprotein profile, high density lipoprotein subclass, and postheparin lipases during gestation in women. J Lipid Res. 1996 Feb;37(2):299-308.

13. Preda A, Preda SD, Mota M, Iliescu DG, Zorila LG, Comanescu AC, Mitrea A, Clenciu D, Mota E, Vladu IM. Dyslipidemia in Pregnancy: A Systematic Review of Molecular Alterations and Clinical Implications. Biomedicines. 2024 Oct 3;12(10):2252. doi: 10.3390/biomedicines12102252.

14. Vahedian-Azimi A, Bianconi V, Makvandi S, Banach M, Mohammadi SM, Pirro M, Sahebkar A. A systematic review and meta-analysis on the effects of statins on pregnancy outcomes. Atherosclerosis. 2021 Nov;336:1-11. doi: 10.1016/j.atherosclerosis.2021.09.010. Epub 2021 Sep 10.

15. Lewek J, Bielecka-Dąbrowa A, Toth PP, Banach M. Dyslipidaemia management in pregnant patients: a 2024 update. Eur Heart J Open. 2024 Apr 26;4(3):oeae032. doi: 10.1093/ehjopen/oeae032. PMID: 38784103.

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HYPERBILIRUBINEMIA AS A BIOMARKER FOR COMPLICATED APPENDICITIS: A RETROSPECTIVE STUDY

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Abstract

Introduction: Hyperbilirubinemia has been proposed as a potential biomarker for predicting complicated appendicitis. This study aims to evaluate the role of serum bilirubin in differentiating complicated from uncomplicated appendicitis and to compare its diagnostic performance with other inflammatory markers.

Material and Method: A retrospective analysis was conducted on 30 patients diagnosed with acute appendicitis, including 10 with complicated and 20 with uncomplicated appendicitis. Laboratory parameters, including white blood cell (WBC) count, neutrophil-to-lymphocyte ratio (NLR), platelet (PLT) count, interleukin-6 (IL-6) and serum bilirubin levels, were compared between the two groups. Statistical analyses were performed to determine the predictive value of hyperbilirubinemia for complicated appendicitis.

Results: Patients with complicated appendicitis exhibited significantly higher serum bilirubin levels than those with uncomplicated appendicitis ($p<0.01$). Additionally, CRP and IL-6 were notably elevated in the complicated appendicitis group. However, the combination of serum bilirubin with other inflammatory markers, such as CRP and IL-6, enhanced diagnostic accuracy.

Conclusion: Hyperbilirubinemia is a valuable biomarker for predicting complicated appendicitis and may serve as a useful adjunct to existing diagnostic tools. Further large-scale prospective studies are warranted to validate these findings and establish standardized cutoff values for clinical application.

Key Words: *biomarker; complicated appendicitis; serum bilirubin.*

Introduction

Acute appendicitis is one of the most common causes of acute abdomen requiring emergency surgical intervention. It is estimated that approximately 7-8% of the population will develop appendicitis during their lifetime, with the highest incidence occurring in adolescents and young adults (1). Despite significant advancements in diagnostic and therapeutic approaches, acute appendicitis remains a challenge due to its variable clinical presentation and potential for

rapid progression to complications. From a pathophysiological perspective, appendicitis can be classified into two main categories: 1. uncomplicated appendicitis, characterized by localized inflammation without perforation or gangrene, usually having a favorable outcome with timely intervention; and 2. complicated appendicitis, which involves gangrene, perforation, or abscess formation and is associated with higher morbidity, prolonged hospital stay and an increased risk of postoperative complications. Early differentiation between these forms is essential for determining treatment and preventing complications. Although clinical scoring systems (e.g. Alvarado) and imaging techniques significantly contribute to diagnosis, they are not always sufficiently sensitive or specific in predicting the severity of the inflammatory process (2). Researchers have explored the role of biomarkers such as serum bilirubin, inflammatory cytokines, and hematological indices in distinguishing between uncomplicated and complicated appendicitis.

Hyperbilirubinemia has been proposed as a potential marker for complicated appendicitis, as increased serum bilirubin levels may result from bacterial translocation and endotoxemia, leading to hepatocellular dysfunction and reduced bilirubin clearance (3). Studies have reported that patients with perforated appendicitis exhibit significantly higher bilirubin levels compared to those with uncomplicated cases, suggesting thier utility as a predictor of disease severity (4).

In addition to bilirubin, inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), have been investigated for their role in appendicitis severity. IL-6, a key pro-inflammatory mediator, has shown to correlate with disease progression, with higher levels observed in patients with gangrenous or perforated appendicitis (5). Moreover, hematological indices, such as the neutrophil-to-lymphocyte ratio (NLR) and platelet count (PLT), have gained attention as cost-effective and easily accessible markers. Studies suggest that an elevated NLR is associated with a higher likelihood of complicated appendicitis, reflecting a heightened systemic inflammatory response. Integration of these biomarkers into clinical practice could enhance diagnostic accuracy, especially in borderline cases where imaging findings are inconclusive. While further large-scale prospective studies are needed to validate their predictive value, current evidence suggests that serum bilirubin, IL-6, NLR and other inflammatory markers hold promise in improving early risk stratification for complicated appendicitis (3,4,5).

The aim of this study is to evaluate the diagnostic and prognostic potential of serum bilirubin levels in comparison to WBC, NLR, PLT and IL-6 for preoperative identification of complicated appendicitis. Confirming its predictive value could contribute to improving diagnostic strategies and the early identification of high-risk patients.

The primary objective of this study is to investigate the diagnostic and prognostic value of serum bilirubin in distinguishing complicated from uncomplicated appendicitis. Secondary objectives of this study are: 1. to compare the mean values of these biomarkers between patients with complicated (n=10) and uncomplicated appendicitis (n=20); 2. to analyze the correlation between serum bilirubin levels and inflammatory markers (WBC, NLR, PLT, IL-6); and 3. to assess the sensitivity and specificity of each biomarker, individually and in combination, for predicting complications.

Material and Method

This study is designed as a retrospective, observational, monocentric study conducted in the period from January 2023 to January 2024 at the General Hospital Kumanovo. A total of 30 pa-

tients hospitalized with a diagnosis of acute appendicitis and undergoing surgical treatment (either open or laparoscopic appendectomy) were included. Patients were divided into two groups based on histopathological findings:

- **complicated appendicitis group (n=10):** patients with gangrenous or perforated appendicitis or the presence of an abscess; and
- **uncomplicated appendicitis group (n=20):** patients with catarrhal or phlegmonous appendicitis without evidence of complications.

Inclusion Criteria:

- patients over 14 years old diagnosed with acute appendicitis,
- diagnosis confirmed through histopathological analysis after appendectomy, and
- availability of biochemical data, including WBC, NLR, PLT, IL-6, CRP and serum bilirubin.

Exclusion Criteria:

- patients with chronic liver diseases, hemolytic disorders, or previously diagnosed with Gilbert's syndrome,
- pregnant women and patients with confirmed viral hepatitis infection; and
- incomplete medical records in the patient's documentation.

From medical records, demographic data (age, gender) and the following laboratory parameters were collected: WBC (white blood cell count), NLR (neutrophil-to-lymphocyte ratio), PLT (platelet count), IL-6 (interleukin-6), CRP (C-reactive protein) and total serum bilirubin.

Results

Table 1.

	gender	age	WBC	NLR	PLT	CRP	TBIL	IL-6	PHA
1	F	20	19	15.81	250	105.1	12.7	69.3	phleg
2	F	47	14.7	9.9	171	146.5	18.3	89.9	gang
3	M	64	18.9	9.05	358	132.7	53	63.6	gang
4	M	42	8.8	5.92	140	81.8	25	5.76	phleg
5	F	26	21.5	9.4	343	69.2	18	13.3	gang
6	F	44	10.3	4.39	324	102.2	9	6.51	phleg
7	M	47	12.8	16.71	204	11.6	14.3	26.1	phleg
8	F	48	18.7	29.5	227	41.7	15	59.6	phleg
9	F	34	12.2	8.67	195	6.1	7	8.66	phleg
10	F	26	10.8	5.87	218	43.9	9.1	2.3	phleg
11	M	21	11.9	2.86	314	18.1	7.5	4.36	phleg
12	M	33	8.2	3.81	221	19.8	16	3.4	phleg

	gender	age	WBC	NLR	PLT	CRP	TBIL	IL-6	PHA
13	F	39	18.1	7.34	249	0.5	9	27.2	phleg
14	F	15	8.9	4.67	306	27.4	26	2.05	phleg
15	M	52	4.1	6.8	114	47.3	11	46.2	phleg
16	M	18	15.1	4.76	190	0.3	8	12.3	phleg
17	F	29	7.9	2.36	182	0.3	25	2.1	phleg
18	M	24	19.8	11	216	21.1	20.5	92.9	gang
19	F	16	7.4	5.45	154	107	26.7	8.05	phleg
20	F	52	15.6	5.82	214	34.3	13	40.3	gang
21	M	77	19.9	23.14	254	165.4	20	282	phleg
22	M	24	12.8	6.63	204	6.8	23	18.8	gang
23	M	25	4.2	3.44	188	162.3	32	563	gang
24	M	14	21	12.6	342	149.8	28	48.3	gang
25	M	31	13.9	5.65	253	123.5	12.7	10.7	gang
26	M	23	12.3	16.29	318	62.2	13	25.8	phleg
27	M	37	9.5	6.58	124	1.9	12	37.4	phleg
28	M	28	12.1	8.33	190	18.5	16.8	25.1	phleg
29	M	22	26.1	11.6	334	85	26.6	93.8	gang
30	F	50	10.8	3.43	230	14.4	6	6.23	phleg

Table 1 presents the demographic data (age, gender (M – male and F – female)) and the values of the following laboratory parameters (denoted by their respective abbreviations): white blood cell count x10^9/L (WBC), neutrophil-to-lymphocyte ratio (NLR), platelet count x10^9/L (PLT), C-reactive protein in mg/L (CRP), total bilirubin in μmol/L (TBIL), and interleukin-6 in pg/mL (IL-6). The pathological finding (PHA) is categorized into two groups: uncomplicated (phleg) and complicated (gang).

Table 2.

	age	WBC	NLR	PLT	CRP	TBIL	IL-6
count	30.00	30.00	30.00	30.00	30.00	30.00	30.00
mean	34.27	13.58	8.93	234.23	60.22	17.81	56.50
std	15.35	5.38	6.17	68.44	54.75	9.83	109.85
min	14.00	4.10	2.36	114.00	0.30	6.00	2.05
0.25	23.25	9.70	4.93	190.00	15.33	11.25	6.90
0.50	30.00	12.55	6.72	219.50	42.80	15.50	25.45
0.75	46.25	18.55	10.75	293.00	104.38	24.50	56.78
max	77.00	26.10	29.50	358.00	165.40	53.00	563.00

Table 2 presents the basic statistical parameters (mean, standard deviation, minimum and maximum values) for age, WBC, NLR, PLT, CRP, TBIL and IL-6 in patients with appendicitis.

Table 3.

	age		WBC		NLR		PLT		CRP		TBIL		IL-6	
	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std
gang	32.90	15.91	16.85	6.04	8.51	2.98	262.30	73.77	93.12	57.81	24.51	11.81	103.46	164.68
phleg	34.95	15.44	11.94	4.30	9.13	7.34	220.20	62.84	43.78	46.24	14.46	6.78	33.02	61.89

Table 3 compares the mean values and standard deviations (std) for the two patient groups (uncomplicated (phleg) and complicated appendicitis (gang)).

Table 4.

	Test Type	p-value
age	Mann-Whitney U test	0.72
WBC	T-test	0.04
NLR	Mann-Whitney U test	0.39
PLT	T-test	0.14
CRP	Mann-Whitney U test	0.02
TBIL	Mann-Whitney U test	0.01
IL-6	Mann-Whitney U test	0.01

Table 4 presents the p-values from the Mann-Whitney U test and T-test to determine the statistical significance of differences between the groups.

Table 5.

	age	WBC	NLR	PLT	CRP	TBIL	IL-6
age	1.00	0.06	0.32	-0.12	0.19	0.05	0.16
WBC	0.06	1.00	0.55	0.58	0.20	0.14	-0.05
NLR	0.32	0.55	1.00	0.17	0.24	0.02	0.16
PLT	-0.12	0.58	0.17	1.00	0.28	0.25	-0.07
CRP	0.19	0.20	0.24	0.28	1.00	0.49	0.55
TBIL	0.05	0.14	0.02	0.25	0.49	1.00	0.34
IL-6	0.16	-0.05	0.16	-0.07	0.55	0.34	1.00

Table 5 presents the correlations between different laboratory parameters, showing which markers are the most strongly associated.

Table 6.

		Spearman Correlation	p-value
WBC	NLR	0.6918	0.0000
WBC	PLT	0.5920	0.0006
WBC	IL-6	0.5532	0.0015
NLR	WBC	0.6918	0.0000

		Spearman Correlation	p-value
NLR	IL-6	0.6570	0.0001
PLT	WBC	0.5920	0.0006
CRP	TBIL	0.4925	0.0057
CRP	IL-6	0.4563	0.0113
TBIL	CRP	0.4925	0.0057
IL-6	WBC	0.5532	0.0015
IL-6	NLR	0.6570	0.0001
IL-6	CRP	0.4563	0.0113

Table 6 shows the Spearman correlation of numerical variables.

Discussion

This study investigated the diagnostic and prognostic value of serum bilirubin as a potential biomarker for preoperative identification of complicated appendicitis. The results showed that patients with complicated appendicitis (gangrenous/perforated) had significantly higher serum bilirubin levels compared to those with uncomplicated appendicitis. These findings suggest that bilirubin may be a useful indicator for identifying high-risk patients.

According to the analysis, patients with gangrenous appendicitis had higher mean values of WBC, CRP, TBIL, and IL-6 compared to those with phlegmonous appendicitis. The most significant differences were observed in CRP ($p = 0.02$), TBIL ($p = 0.01$), and IL-6 ($p = 0.01$), indicating that these biomarkers play a crucial role in distinguishing complicated appendicitis.

The strong correlation between TBIL and CRP ($\rho=0.49$, $p=0.0057$) supports the hypothesis that increased bilirubin levels are associated with an elevated inflammatory response. This may be explained by the effect of bacterial endotoxemia on bilirubin metabolism, leading to its elevation in patients with severe inflammation.

These findings are consistent with previous research, which has shown that hyperbilirubinemia is an indicator of complicated appendicitis (4,6,7). Studies conducted by Gavriilidis et al. (2019) and Burchart et al. (2013), also suggest that elevated bilirubin levels may signal gangrene or perforation of the appendix (8,9). However, larger studies are needed to further validate these results (10).

Some limitations of this study include a small patient sample (n=30), which reduces the statistical power of the analysis. Additionally, this is a retrospective study, meaning biases related to data collection are possible. Future research with a larger sample size and a prospective design could confirm these findings.

Conclusion

The results of this study indicate that serum bilirubin may serve as a useful biochemical marker for the preoperative identification of complicated appendicitis. Its application, in combination with traditional inflammatory parameters (CRP, WBC, IL-6), could improve diagnostic strate-

gies and optimize surgical treatment.

References:

1. Bhangu A, Soreide K, Di Saverno S, et al. Acute appendicitis: modern understanding of pathogenesis, diagnosis, and management. Lancet. 2015; 386:1278-87.
2. Arruzza E, Milanese S, Li LSK, et al. Diagnostic accuracy of computed tomography and ultrasound for the diagnosis of acute appendicitis: a systematic review and meta-analysis. Radiography. 2022; 28:1127-1141.
3. Koirala DP, Gupta AK, Dahal GR, et al. Role of hyperbilirubinemia as a predictor of complicated appendicitis in pediatric population. Afr J Paediatr Surg. 2022; 19(2):61-64.
4. Giordano S, Paakkonen M, Salmien P, et al. Elevated serum bilirubin in assessing the likehood of perforation in acute appendicitis: a diagnostic meta-analysis. Intl J Surg. 2013; 11:795-800.
5. Binboga S, Isiksacan N, Binboga E, et al. Diagnostic value of serum cytokines in predicting a complicated acute appendicitis. An Acad Bras Cienc. 2022; 94(2):e20201947.
6. Pogorelic Z, Luksic AM, Mihanovic J, et al. Hyperbilirubinemia as an indicator of acute appendicitis in pediatric population: a prospective study. Surg Infect. 2021; 22(10):1064-1071.
7. Haider Kazmi SJ, Tabassum S, Ashgar MS, et al. Role of inflammatory and hematological markers in predicting the complicated and perforate appendicitis in pediatric population: a cohort study. Annals of Medicine and Surgery. 2022; 76:103524.
8. Gavriilidis P, de Angelis N, Evans J, et al. Hyperbilirubinemia as a predictor of appendiceal perforation: a systematic review and diagnostic test meta-analysis. J Clin Med Res. 2019; 11(3):171-178.
9. Burchart J, Pommergaard HC, Rosenberg J, et al. Hyperbilirubinemia as a predictor for appendiceal perforation: a systematic review. Scand J Surg. 2013; 102(2):55-60.
10. Khalid SY, Elamin A. The diagnostic accuracy of hyperbilirubinemia in predicting appendicitis and appendiceal perforation. Cureus. 2023; 15(11):e48203.

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INVESTIGATION OF DEXMEDETOMIDINE, FAMOTIDINE AND METOCLOPRAMIDE EFFICACY FOR PREVENTION OF POSTOPERATIVE NAUSEA AND VOMITUS: WHAT IS THE PREFERABLE CHOICE?

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Abstract

Introduction: Postoperative nausea and vomitus (PONV) are serious complication subsequent to laparoscopic radical prostatectomy. Although the prevention of PONV states that a single bolus dose of one antiemetic drug is recommended, combining treatment with two or more drugs or continuous antiemetic drug infusion is more effective.

Objectives: To evaluate and compare the occurrence and severity of postoperative nausea and vomiting (PONV) in the postoperative phases in patients undergoing laparoscopic prostatectomy who receive various antiemetic prophylaxes.

Materials and Methods: This prospective, comparative study included 40 patients who underwent laparoscopic radical prostatectomy, equally divided into two groups: one receiving intraoperative antiemetic prophylaxis with a combination of two agents Group FM (intra operative receiving metoclopramide and famotidine), and the other with a single agent (receiving intra- and postoperative continuous infusion of Dexmedetomidine) Group DEX. The dry retching and nausea were assessed at five postoperative time points: immediately after extubating, and at 2, 4, 12 and 24 hours.

Results: Postoperatively, PONV was identified in total 8 (20%) patients in the two groups. The incidence of PONV in group DEX was lower, and it was 15% or 3 patients with grade 1 PONV, versus 25% or 5 patients with grade 2 PONV in group FM.

Conclusion: Our results indicate that Dexmedetomidine could significantly lower the occurrence and gradus of PONV compared, with combination of Famotidine and Metoclopramide after laparoscopic prostatectomy.

***Key Words**: dexmedetomidine; metoclopramide; postoperative nausea and vomitus.*

Introduction

Over the years, surgical techniques have evolved significantly, with minimally invasive approaches like laparoscopic prostatectomy (LPR) becoming increasingly common. Compared to open surgery, LPR offers several advantages: reduced trauma, less postoperative pain and feasibility as a day-case procedure (1).

However, the creation of pneumoperitoneum using carbon dioxide can cause notable hemodynamic, renal and respiratory effects due to elevated intra-abdominal pressure (2). The occurrence of postoperative nausea and vomiting (PONV) as one of the most common postoperative complications of LPR has incidence of 40–70% (3). This is largely triggered by CO_2 insufflation, which activates serotonin receptors in the gut and stimulates the chemoreceptor trigger zone (4). Risk factors for PONV include non-smoking patients, general anesthesia, laparoscopic interventions, history of motion sickness and stress-prone personality (5).

Despite modern antiemetic strategies, PONV remains one of the most frequent complications in the first 24 hours after laparoscopic surgery (6,7). It can lead to prolonged recovery, delayed discharge, increased healthcare costs, and complications such as appetite loss, dehydration, electrolyte imbalance, wound dehiscence, esophageal rupture and even pneumothorax (8,9). The incidence rises to 53–70% in high-risk patients (Apfel score ≥3) (9). Therefore, crucial for improved patients' outcomes and reducing hospital stay costs, is early prevention and treatment of PONV.

Monitoring PONV is especially important in high-risk groups, influenced by factors like gender, surgery type and duration, anesthesia duration, CO_2 exposure, and even emotional stress in the recovery room (10). The vomiting reflex is mediated through the vomiting center and the chemoreceptor trigger zone (CTZ) in the medulla oblongata (11). Other risk factors include age >50, female gender, infections, uremia, migraines, hypercalcemia and anxiety (9,10,11). Specific surgeries such as abdominal laparoscopy, gynecologic procedures, strabismus, and ear surgery are also associated with higher PONV rates (10,11).

Prophylaxis depends on risk level. Single-drug therapy is recommended for moderate-risk patients (Apfel score 1–2), while high-risk patients benefit more from combination therapies involving multiple antiemetic drug classes (10). The most common used drugs are: butyrophenones, serotonin antagonists, steroids, H_2-receptor antagonists, anticholinergics and phenothiazines. Famotidine, metoclopramide and dexmedetomidine are among the drugs used. Although famotidine has no direct antiemetic effect, it inhibits histamine H_2 receptors in gastric parietal cells, reducing acid secretion. Given intravenously, it reaches peak effect in 30 minutes and lasts 10–12 hours (12). Metoclopramide, a dopamine receptor antagonist with prokinetic effects, acts both centrally and peripherally. Its onset is within 15 minutes and duration 1–2 hours (13). Dexmedetomidine, an α_2-agonist, exerts antiemetic effects by reducing sympathetic tone and perioperative opioid use. It begins acting within 5–10 minutes, peaks at 15–30 minutes, and lasts 60–120 minutes. With a half-life of 2 hours, its pharmacokinetic profile supports use in both intraoperative and early postoperative settings (14).

Hence, the main objective of this small study was to compare the prophylactic effects of famotidine and metoclopramide combination alongside dexmedetomidine in reducing PONV in patients undergoing laparoscopic prostatectomy.

Materials and Methods

Study Design: This study was designed as a prospective comparative clinical evaluation conducted at the University Clinic of Urology, Skopje, and the University Clinic for Anesthesiology, Reanimation and Intensive Care Medicine Faculty of Medicine, "Ss Cyril and Methodius" University, Skopje, RN Macedonia. The evaluation included a total of 40 male patients scheduled for laparoscopic prostatectomy (LPR) between January and December 2024. The enrollment of the patients scheduled for LPR in the study was conducted after obtaining informed consent. The study protocol received ethical approval from the Institutional Review Board (IRB) and ethical committee from the medical faculty, and written informed consent was obtained from all participants before inclusion in the investigation.

Patients' Selection Criteria: Inclusion criteria incorporated male patients aged 50–75 years with histologically confirmed prostate carcinoma requiring LPR, ASA Class I and II and 180-minutes maximum duration of surgery. Patients with pre-existing psychiatric illnesses, Parkinson's disease, motion sickness, or a history of chemotherapy were excluded to minimize confounding factors affecting PONV. Excluding factors were identified allergies to the medications in this study but also patients with EF ≤30%, coronary occlusions ≥ 50%, bradycardia ≤ 50 min, MAP ≤ 65mmHg and atrioventricular block grade II, due to the use of Dexmedetomidine.

The patients were randomly divided into two groups (combination of famotidine and metoclopramide - group FM and dexmedetomidine - group DEX) using a cubull randomization. All patients received complete monitoring, including noninvasive blood pressure (NIBP), heart rate (PR), oxygen saturation (SpO_2) and body temperature measurements.

Group FM (Famotidine + Metoclopramide): Patients in this cohort were given 20mg of famotidine and 10mg of metoclopramide intraoperatively, immediately following intubation. Group DEX (Dexmedetomidine): Patients in this cohort were administered dexmedetomidine at a dosage of 0.4 micrograms per kilogram per hour (μg/kg/h) during the intraoperative phase, thereafter followed by a decreased dosage of 0.1μg/kg/h for 8 hours postoperatively.

Examined Parameters: PONV was assessed through serial of physical examinations and questionnaires in five different time points:

- T1 - Immediately after the extubating,
- T2 - 2 h after the surgery,
- T3 - 4 h after the surgery,
- T4 - 12 h after the surgery,
- T5 - 24 h after the surgery.

After the completion of surgery, the first physical exam and questionnaire for T1 was taken in the operating room, after which the patients entered the recovery room where the following checkups and surveys, including questions about the scour of nausea and vomiting and hemodynamic parameters of the patient in T2, T3 and T4 were completed. The last exam and questionary for T5 were taken in the patient's room. All patients with vomiting scores of 2 and > 5 were treated with ondansetron *(*4mg I.V. 1cc). Ondansetron is one of the imperative drugs in preventing PONV due to surgery and chemotherapy. This serotonin receptor antagonist exhib-

its its anti-vomiting effects by inhibiting 5-hydroxytryptamine type 3 (5-HT3) receptors in the vomiting center and the compressor starting area (15). Finally, the obtained data were analyzed by statistical software SPSS 23 and the data were presented in the form of statistical tables and charts.

Assessment of PONV: Evaluation of the risk for PONV was made with use of the Apfel risk score and determining the grade and severity of PONV was made using the most recent grade and impact scale respectively.

Table 1. Apfel's risk score and the PONV impact scale.

Apfel's risk factors	
Non-smoker	1
Postoperative opioids	1
History of PONV	1
Female gender	1
Total score	0-4 (Total score ≥ 3 clinically significant)
PONV impact scale calculator	
Dry-retching episodes	
Not at all	0
Once	1
Twice	2
Three or more times	3
Nausea episodes	
Not at all	0
Sometimes	1
Often or most of the time	2
All the time	3
Total score (≥5 clinically significant)	0-6

One of the tools that have proven to be effective in assessing the patient's baseline risk PONV and also has implications in the protocol for patient-specific antiemetic prophylaxis is the Apfel's risk score. The factors included in the Apfel's score are postoperative use of opioids, non-smoker status, female gender and previous history of PONV or motion sickness. Correspondingly, all these risk factors contribute to elevating the incidence of PONV by about 20% (9). Each risk factor is given a score of 1, the total score being 4. PONV is classified as grades 0, 1 and 2. Grades 1 and 2 are considered as PONV (16).

PONV impact scale calculator is a tool that assesses the clinical significance of the PONV, and it is based on the patient's assessment of the impact of their nausea on their postoperative recovery and the number of experienced vomiting. It includes questions about the presence of nausea and its quantity and questions about presence and the number of vomiting. A score ≥5 is considered clinically significant (16).

Table 2. PONV grade.

PONV grade	Patient's response
0	Without PONV
1	Nausea without vomitus
2	Nausea with vomiting (≤ 3 times/day)
3	Vomiting ≥ 3 times /day

PONV grade is determined by a four-point (0-3) scoring system, with PONV score 0= no signs of nausea and retching; 1= episodes of sickness and retching; 2= vomiting one or two times in a period of 30 min; 3= vomiting more than two times in a period of 30 min (9,16).

Anesthesia Protocol: Per the protocol, all patients received conventional preoperative preparation, which included a minimum fasting period of six hours and the maintenance of normothermia. Two hours before surgery, patients received 5mg of oral diazepam as premedication. Upon entering the operating room, patients were subjected to continuous hemodynamic monitoring utilizing the Datex-Ohmeda S/5 Avance (Helsinki, Finland), which recorded the following parameters: electrocardiography (ECG), heart rate (HR), non-invasive blood pressure (NIBP) and invasive mean arterial pressure (MAP) at five-minute intervals, along with oxygen saturation (SpO_2), capnography (end-tidal CO_2 – $EtCO_2$), fraction of inspired oxygen (FiO_2), and intra-abdominal pressure (9–12 mmHg) through the laparoscopic insufflation system. An intravenous cannula was inserted in each patient, and a crystalloid infusion was delivered at a rate of 6–12ml/kg/hr during anesthesia. Before induction, patients received preoxygenation with 100% oxygen at a flow rate of 6 L/min for three minutes. General endotracheal anesthesia was initiated with 0.04mg/kg midazolam, 0.002mg/kg fentanyl, 1–2mg/kg propofol, and 0.6mg/kg rocuronium. After loss of consciousness and the stoppage of spontaneous respiration, patients were manually ventilated, and endotracheal intubation was conducted two minutes post-administration of rocuronium. Mechanical ventilation commenced utilizing the Datex-Ohmeda S/5 Avance in Pressure-Controlled Ventilation - Volume Guarantee (PCV-VG) mode, with a tidal volume of 6–8ml/kg, a gas mixture comprising 50% oxygen and 50% air, an inspiratory-to-expiratory ratio (I:E) of 1:2, a respiratory rate calibrated to sustain $EtCO_2$ between 35–45mmHg, and a positive end-expiratory pressure (PEEP) of 5cm H_2O. Anesthesia was sustained by a balanced method utilizing remifentanil (0.05–1μg/kg/min) and sevoflurane at 1 MAC, ensuring mean arterial pressure remained within ±20% of baseline values. A nasogastric tube was inserted for decompression, intraoperative normothermia was sustained using forced-air warming blankets, and anti-embolism pumps were utilized for all patients to avert thromboembolic problems. Postoperative treatment encompassed standardized analgesia and fluid resuscitation according to institutional procedure (2024 NICE guidelines). 25-30ml/kg/day of water and 1mmol/kg/day of sodium, potassium, and chloride, in accordance to the British Consensus Guidelines on IV Fluid for Adult Surgical Patients. GIFTASUP advised a low volume maintenance fluid of 1-1.5ml/kg/hr, with fluid boluses of 0.5ml/kg/hr for the resuscitation of postoperative oliguria, along with serial evaluations of postoperative nausea and vomiting (PONV) using the PONV grade and impact scale for up to 24 hours postoperatively. Patients suffering from PONV were treated in accordance to the most recent recommendations and guidelines, employing a multimodal approach with antiemetic medications, and utilizing ondansetron as the "gold standard" for PONV management (17).

All patients with vomiting scores of 2 and > 5 were treated with *ondansetron* 4mg i.v. /1cc.

Statistical Analysis: The data were examined utilizing SPSS software (version 27.0, IBM Corp.). Continuous variables were assessed for normalcy with the Shapiro-Wilk test. Parametric data were represented as mean ± standard deviation (SD) and evaluated via the paired t-test, whereas non-parametric data were provided as a median with interquartile range (IQR) and compared using the Mann-Whitney U test. A p-value of less than 0.05 was deemed statistically significant.

Results

Out of a total of 40 patients who underwent laparoscopic prostatectomy, 3 patients (15%) from the DEX group had Apfel score of 3 with a 60% possibility of developing PONV, and from the FM group, one patient (5%) had Apfel score of 3, which means a 60% possibility of PONV, and one patient from the same group had an Apfel score 4 (5%) with an 80% possibility of PONV. From those patients with significant predictive Apfel score from the FM group (from the PONV impact scale calculator), 5 patients had PONV in T1, 4 patients in T2, 2 patients in T3, 1 patient in T4, and no patients in T5. Furthermore, out of these patients, 1 had grade 0 PONV, 2 had grade 1 PONV and 2 had grade 2 PONV. The number of patients with significant predictive Apfel score from the DEX group (score ≥5 from the PONV impact scale calculator) was 3 patients in T1, 1 patient in T2, and no patients in T3, T4 and T5. Out of these patients, 2 had grade PONV 1.

Table 3 presents the demographic characteristics of the study population: a mid-age value of 61 (50-72) years in the FM group and 65 (55-75) in the DEX group. Average BMI of 30 (21-39) kg/m^2 in the FM group and 31 (29-32) kg/m^2 in the DEX group. The average duration of LRP in the FM group was 155 (135-175) minutes and 150 (138-172) minutes in the DEX group. There were 6 patients, or 30%, who were non-smokers in the FM group and a total of 3 non-smoker patients in the DEX group. According to ASA score there were 14 patients (70%) with an ASA I score in the FM group and the same number of 14 (70%) ASA I patients in the DEX group. The number of ASA II patients in both groups was also the same, 6 (30%). ASA III and ASA IV patients were excluded from the study.

Table 3. Patients' demographic characteristic (N=40).

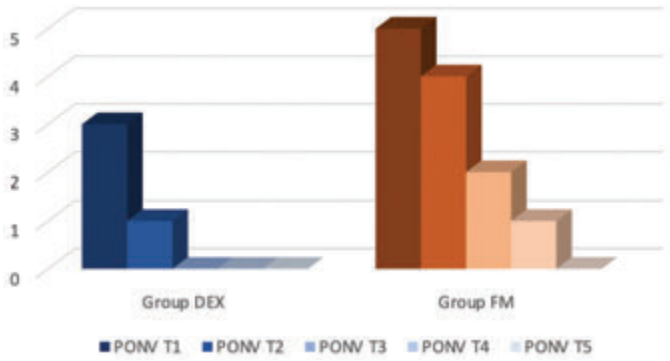
Parameter	Famotidine + Metoclopramide Group FM n(%)	Dexmedetomidine Group DEX n(%)
Age (years), median (IQR)	61 (50-72)	65 (55-75)
BMI (kg/m2), median (IQR)	30 (21-39)	31 (29-32)
Average duration of surgery(min), median (IQR)	155 (135-175)	150 (138-172)
Nonsmoker	6 (30)	3(15)
ASA score		
I	14(70)	14(70)
II	6(30)	6(30)
III	0(100)	0(100)
IV	0(100)	0(100)
Previous PONV	4 (20)	3(15)
Previous motion sickness	3(15)	2(10)
Apfel score		

1	4(20)	0(100)
2	3(15)	0(100)
3	1(5)	3(15)
4	1(5)	0(100)

There were 4 (20%) patients with previous PONV in the FM group and 3 (15%) in the DEX group. Previous motion sickness was noted in 3 patients (15%) in the FM group and in 2 patients (10%) in the DEX group.

Apfel score for predicting the PONV was 1 for 4 patients (20%) in the FM group and none in the DEX group. Apfel score 2 for 3 (15%) of patients in the first and none in the latter group. Apfel score 3 was noted in 1 patient (5%) in the FM, versus 3 (15%) patients in the DEX group. And finally, Apfel score 4 was noted just in 1 (5%) patient in the first FM group.

The difference between the two groups regarding mean age, BMI and mean duration of surgery was noteworthy ($P \geq 0.05$).



Graph 1. Comparing vomiting and nausea scores in T1, T2, T3, T4, T5 of DEX group versus FM group.

Giving the results in Graph 1 there was a substantial difference in the frequency and the grade of nausea and vomiting among the two groups, and the incidence of PONV exhibited a significant decrease in the DEX group as compared to FM groups ($p<0.005$).

Table 4. Comparison of the incidence and severity of PONV between the two groups.

Group DEX	Group FM
PONV T1 = 3	PONV T1 = 5
PONV T2 = 1	PONV T2 = 4
PONV T3 = 0	PONV T3 = 2
PONV T4 = 0	PONV T4 = 1
PONV T5 = 0	PONV T5 = 0
Grade 0 = 0	Grade 0 = 1
Grade 1 = 2	Grade 1 = 2
Grade 3 = 0	Grade 2 = 2

The incidence of PONV in the DEX group was lower and it was 15 % with grade 1 versus 25 % with grade 2 in the FM group.

Discussion

Anesthesiologists play a crucial role in determining an appropriate pharmacological regimen for managing PONV. One of the symptoms of PONV that could occur during the first 24 hours after general anesthesia, nausea, is defined as a feeling of unpleasant agitation and discomfort in the abdomen, followed by inevitable occurrence of vomiting (18). Despite the certain advancements in the field of new drugs for PONV, nausea and vomiting are still persistent as a common complaint after general anesthesia, with frequency of occurrence from 20% to 30% of the patients who undergo general anesthesia within 24 hours of surgery (18, 19). The incidence of nausea and vomiting post-surgery depends upon numerous circumstances, including the surgical procedure (laparoscopy, strabismus correction, ear surgery, gynecological surgery), the anesthetic drugs, and also the anesthetic employed in the procedure (17,18,19).

Laparoscopic prostatectomies are now increasingly being performed. Shorter hospital stay is the advantage of this procedure, but PONV may lengthen stay in hospital and increase the treatment cost. The results of previous studies were in accordance to our study.

The research by Masilamani involving 100 patients revealed an average hospital stay of 1.19 days, much lower than the 3-4 days typically required for open radical prostatectomy (20). Previous research by Parra-Sanchez et al., demonstrated that patients experiencing PONV had a significantly prolonged stay in the post-anesthesia care unit compared to those without PONV. This supports the notion that PONV not only affects patient's comfort but also has a measurable impact on recovery efficiency and resource utilization. Furthermore, there was meaningfully a notable difference in the nursing time required for patients with PONV than the patients without PONV with statistically significant numbers. Subsequently, the total cost of postoperative recovery for PONV patients was greater and therefore was associated with an adjusted incremental total cost. The postoperative quality of life in PONV patients was worse (49% of patients with PONV rated quality high in four domains vs 94% of patients without PONV (21). These results align with those obtained in our investigation.

Prevention and treatment of PONV alongside providing suitable scales and drugs has been one of the important concerns of anesthesiologists over the years. The drugs that are used as prophylaxis or for the treatment of PONV include serotonin antagonists, anticholinergics, butyrophenones, phenothiazines, steroids, and histamine H2-receptor antagonists. Although the recommendations stand for a single-drug prophylactic administration, combining treatment with two or more drugs from different classes or continuous infusion of anti-emetic drugs is more effective than single medicine for high-risk patients (4,10). The results from using some of these anti-emetic drugs: famotidine, metoclopramide and dexmedetomidine —in our study were in agreement with previous studies, like the study of Nesek-Adam V. that included 160 patients in which none of the patients form the dexamethasone plus metoclopramide group patients ($p< 0.05$ versus groups 1 and 2) and only one of the dexamethasone group patient ($p< 0.05$ versus group 1) required antiemetic rescue, vice the four patients in the metoclopramide group and six patients in the placebo group that and PONV (1). Another study also contributed to the results that the combination of two antiemetic drugs was found to meaningfully decrease the incidence of PONV compared to single antiemetic drug. Furthermore, in a series of 140 patients the results were as follows: significantly lower rate of PONV in patients receiving a combination of metoclopramide and droperidol than those administered metoclopramide alone or placebo. Those receiving two-dose droperidol alone also had a statistically significantly lower incidence

of PONV compared to metoclopramide and placebo (4).

A score to rate clinically important PONV from a patient's point of view was developed and validated by Wengritzsky et coauthors and named the PONV intensity scale. The practicality of the PONV intensity scale led to the development and validation of a simplified score by Myles and Wengritzky, named the PONV impact scale (22). It consists of two questions directed to patient (Table 1). A score of ≥5 from the two questions defines clinically significant PONV. It has been reported that patients perceive PONV to be more distressing than pain, which necessitates assessment of its incidence to ensure that it is not undertreated, and that effective measures are undertaken to address it (9). Weilbach with coauthors, published a prospective study with 93 patients from 2006 that highlighted that in the group with an Apfel score of 3, PONV occurred in 59.7% of the patients and in the Apfel score group of 4, in 91.3% of all patients. The incidence of PONV corresponded to the predicted values of 60% for Apfel 3 and 80% for Apfel 4. The conclusion was that the Apfel score is a useful and simple tool for stratification of patients with high risk for PONV (9, 10, 23).

In terms of which agents have more efficacy and low cost for PONV prophylaxis, more research is required. Our study evidences that there is a significant difference and decrease in the frequency and the grade of nausea and vomiting among the use of dexmedetomidine compared to the combined use of famotidine and metoclopramide ($p<0.005$). It was also confirmed in the meta-analysis with 6,480 patients by the author Liang X. The results confirmed that dexmedetomidine reduces postoperative nausea (Risk Ratio (RR) = 0.61, 95% confidence interval (CI): 0.50 to 0.73) and vomiting compared to placebo, with an effective dose of 0.5μg/kg (RR = 0.46, 95% CI: 0.34 to 0.62) and 1.0μg/kg (RR = 0.29, 95% CI: 0.12 to 0.75), respectively. Moreover, its application lowered intraoperative requirement of fentanyl. The results of this meta-analysis showed the superior dexmedetomidine efficacy to placebo, all related to a reduced intraoperative opioid consumption (14).

Likewase, in an updated meta-analysis trial from 2023 with total 18 trials involving 2018 patients and 15 updated of previous studies, Zhao W et al. supported our findings with the results of PONV incidence in DEX group that is lower than that in the control group (OR=0.49, 95% CI: 0.36 to 0.67), and significantly decreased perioperative opioid consumption in the DEX group (standard mean difference (SMD)=-1.04, 95% CI: -1.53 to -0.54). Moreover, the length of hospitalization (SMD=-2.29, 95% CI: -4.31 to -0.28) and the extubating time (SMD=-0.75, 95% CI: -1.26 to -0.25) in DEX group were shorter. In final conclusion, Dexmedetomidine could decrease the occurrence of PONV in adult patients under general anesthesia and promote the recovery after surgery (24).

Furthermore, in another meta-analysis from 2017, Jin S et al. give more evidence that support our results that dexmedetomidine could decrease the occurrence of PONV after general anesthesia. PONV in the dexmedetomidine group was meaningfully lower versus the placebo group (0.56, 95% CI: 0.46, 0.69). Perioperative fentanyl consumption in the dexmedetomidine group was also reduced significantly ($P<0.00001$). Subgroup analysis showed that dexmedetomidine administration by loading dose plus continuous infusion, by loading dose, or just by continuous infusion, the incidence of PONV during general anesthesia was decreased significantly, and therefore dexmedetomidine administered in continuous infusion mode has the advantage to prevent PONV as well as reducing side effects such as bradycardia and hypotension (25).

This aligns with our findings of significant decrease of the incidence of PONV in the DEX group compared to the FM group ($p<0.005$). The incidence of PONV in the DEX group was lower, and it was 15% with grade 1 versus 25% with grade 2 in the FM group.

Despite the valuable insights gained from this study, one main limitation should be acknowledged: the relatively small sample size that may limit the generalizability of our findings, and larger multicenter studies are needed to confirm these results.

Conclusion

Our findings indicate that the investigated antiemetic drugs (dexmedetomidine, famotidine and metoclopramide) are effective in reducing postoperative nausea and vomiting in patients undergoing laparoscopic prostatectomy. It should be noted, on the other hand, that the antiemetic effect of dexmedetomidine was substantially more powerful when compared to the combination of famotidine and metoclopramide.

References:

1. Nesek-Adam V, Grizelj-Stojcić E, Rasić Z, Cala Z, Mrsić V, Smiljanić A. Comparison of dexamethasone, metoclopramide, and their combination in the prevention of postoperative nausea and vomiting after laparoscopic cholecystectomy. Surg Endosc. 2007 Apr;21(4):607-12. doi: 10.1007/s00464-006-9122-7. Epub 2007 Feb 7. PMID: 17285386.
2. Sodha S, Nazarian S, Adshead JM, Vasdev N, Mohan-S G. Effect of Pneumoperitoneum on Renal Function and Physiology in Patients Undergoing Robotic Renal Surgery. Curr Urol. 2016 Feb;9(1):1-4. doi: 10.1159/000442842. Epub 2016 Feb 10. PMID: 26989363; PMCID: PMC4789951.
3. Szarvas S, Chellapuri RS, Harmon DC, Owens J, Murphy D, Shorten GD. A comparison of dexamethasone, ondansetron, and dexamethasone plus ondansetron as prophylactic antiemetic and antipruritic therapy in patients receiving intrathecal morphine for major orthopedic surgery. Anesth Analg. 2003 Jul;97(1):259-63, table of contents. doi: 10.1213/01.ane.0000066310.49139.2a. PMID: 12818978.
4. Nesek-Adam V, Grizelj-Stojcić E, Mrsić V, Smiljanić A, Rasić Z, Cala Z. Prophylactic antiemetics for laparoscopic cholecystectomy: droperidol, metoclopramide, and droperidol plus metoclopramide. J Laparoendosc Adv Surg Tech A. 2004 Aug;14(4):212-8. doi: 10.1089/lap.2004.14.212. PMID: 15345158.
5. Fujii Y. The utility of antiemetics in the prevention and treatment of postoperative nausea and vomiting in patients scheduled for laparoscopic cholecystectomy. Curr Pharm Des. 2005;11(24):3173-83. doi: 10.2174/1381612054864911. PMID: 16178752.
6. Wu SJ, Xiong XZ, Cheng TY, Lin YX, Cheng NS. Efficacy of ondansetron vs. metoclopramide in prophylaxis of postoperative nausea and vomiting after laparoscopic cholecystectomy: a systematic review and meta-analysis. Hepatogastroenterology. 2012 Oct;59(119):2064-74. doi: 10.5754/hge11811. PMID: 22282127.

7. Kamali, A., Ahmadi, L., Shokrpour, M.,Pazuki, S. Investigation of ondansetron, haloperidol, and dexmedetomidine efficacy for prevention of postoperative nausea and vomiting in patients with abdominal hysterectomy. Open Access Macedonian Journal of Medical Sciences, 2018; 6(9): 1659–1663. https://doi.org/10.3889/oamjms.2018.366 .

8. Eidi M, Kolahdouzan K, Hosseinzadeh H, Tabaqi R. A comparison of preoperative ondansetron and dexamethasone in the prevention of post-tympanoplasty nausea and vomiting. Iran J Med Sci. 2012 Sep;37(3):166-72. PMID: 23115448; PMCID: PMC3470085.

9. Weilbach C, Rahe-meyer N, Raymondos K, Weissig A, Scheinichen D, Piepenbrock S. Postoperative nausea and vomiting (PONV) : usefulness of the Apfel-score for identification of high risk patients for PONV. Acta Anaesthesiol Belg. 2006;57(4):361-3. PMID: 17236637.

10. Darvall J, Handscombe M, Maat B, So K, Suganthirakumar A, Leslie K. Interpretation of the four risk factors for postoperative nausea and vomiting in the Apfel simplified risk score: an analysis of published studies. Can J Anaesth. 2021 Jul;68(7):1057-1063. English. doi: 10.1007/s12630-021-01974-8. Epub 2021 Mar 15. PMID: 33721198.

11. Jabalameli, Mitra et al. "Treatment of postoperative nausea and vomiting after spinal anesthesia for cesarean delivery: A randomized, double-blinded comparison of midazolam, ondansetron, and a combination." *Advanced Biomedical Research* 1 (2012): n. pag.

12. Nozaki C, Morioka N, Kinoshita M, Takada M, Ozaki M. [Can preoperative administration of H2 blocker reduce the incidence of postoperative nausea and vomiting (PONV)?]. Masui. 2007 Oct;56(10):1161-4. Japanese. PMID: 17966618.

13. De Oliveira GS Jr, Castro-Alves LJ, Chang R, Yaghmour E, McCarthy RJ. Systemic metoclopramide to prevent postoperative nausea and vomiting: a meta-analysis without Fujii's studies. Br J Anaesth. 2012 Nov;109(5):688-97. doi: 10.1093/bja/aes325. Epub 2012 Sep 25. PMID: 23015617.

14. Liang X, Zhou M, Feng JJ, Wu L, Fang SP, Ge XY, Sun HJ, Ren PC, Lv X. Efficacy of dexmedetomidine on postoperative nausea and vomiting: a meta-analysis of randomized controlled trials. Int J Clin Exp Med. 2015 Jun 15;8(6):8450-71. PMID: 26309498; PMCID: PMC4538099.

15. Zhang D, Shen Z, You J, Zhu X, Tang QF. Effect of ondansetron in preventing postoperative nausea and vomiting under different conditions of general anesthesia: a preliminary, randomized, controlled study. Ups J Med Sci. 2013 May;118(2):87-90. doi: 10.3109/03009734.2013.768315. Epub 2013 Feb 26. PMID: 23441598; PMCID: PMC3633335.

16. Myles PS, Wengritzky R. Simplified postoperative nausea and vomiting impact scale for audit and post-discharge review. Br J Anaesth. 2012 Mar;108(3):423-9. doi: 10.1093/bja/aer505. Epub 2012 Jan 29. PMID: 22290456.

17. Yoo JH, Jeon IS, Chung JW, Ryoo JH, You GW, Kim SI. Comparison of palonosetron and ondansetron to prevent postoperative nausea and vomiting in women using intravenous patient-controlled analgesia. Anesth Pain Med (Seoul). 2020 Jan 31;15(1):28-34. doi: 10.17085/apm.2020.15.1.28. PMID: 33329786; PMCID: PMC7713856.

18. Rüsch D, Eberhart LH, Wallenborn J, Kranke P. Nausea and vomiting after surgery under general anesthesia: an evidence-based review concerning risk assessment, prevention, and treatment. Dtsch Arztebl Int. 2010 Oct;107(42):733-41. doi: 10.3238/arztebl.2010.0733. Epub 2010 Oct 22. PMID: 21079721; PMCID: PMC2977990.

19. Ghosh S, Rai KK, Shivakumar HR, Upasi AP, Naik VG, Bharat A. Incidence and risk factors for postoperative nausea and vomiting in orthognathic surgery: a 10-year retrospective study. J Korean Assoc Oral Maxillofac Surg. 2020 Apr 30;46(2):116-124. doi: 10.5125/jkaoms.2020.46.2.116. PMID: 32364351; PMCID: PMC7222617.

20. Masilamani MKS, Sukumar A, Cooke PW, Rangaswamy C. Role of multimodal anaesthetic in post-operative analgesic requirement for robotic assisted radical prostatectomy. Urologia. 2022 Feb;89(1):90-93. doi: 10.1177/03915603211031869. Epub 2021 Aug 2. PMID: 34338049.

21. Parra-Sanchez I, Abdallah R, You J, Fu AZ, Grady M, Cummings K 3rd, Apfel C, Sessler DI. A time-motion economic analysis of postoperative nausea and vomiting in ambulatory surgery. Can J Anaesth. 2012 Apr;59(4):366-75. doi: 10.1007/s12630-011-9660-x. Epub 2012 Jan 6. PMID: 22223185.

22. Wengritzky R, Mettho T, Myles PS, Burke J, Kakos A. Development and validation of a postoperative nausea and vomiting intensity scale. Br J Anaesth. 2010 Feb;104(2):158-66. doi: 10.1093/bja/aep370. Epub 2009 Dec 26. PMID: 20037151.

23. Pierre S, Benais H, Pouymayou J. Apfel's simplified score may favourably predict the risk of postoperative nausea and vomiting. Can J Anaesth. 2002 Mar;49(3):237-42. doi: 10.1007/BF03020521. PMID: 11861340.

24. Zhao W, Li J, Wang N, et al. Effect of dexmedetomidine on postoperative nausea and vomiting in patients under general anaesthesia: an updated meta-analysis of randomised controlled trials. BMJ Open. 2023 Aug 1;13(8):e067102. doi: 10.1136/bmjopen-2022-067102. PMID: 37527891; PMCID: PMC10394554.

25. Jin S, Liang DD, Chen C, Zhang M, Wang J. Dexmedetomidine prevent postoperative nausea and vomiting on patients during general anesthesia: A PRISMA-compliant meta analysis of randomized controlled trials. Medicine (Baltimore). 2017 Jan;96(1):e5770. doi: 10.1097/MD.0000000000005770. PMID: 28072722; PMCID: PMC5228682.

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VAGUS NERVE STIMULATOR AS A TREATMENT FOR REFRACTORY EPILEPSY – ONE CENTER EXPERIENCE

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Abstract

Epilepsy is defined as a neurological disorder manifested by an excessive and abnormal electrical neuronal discharge. Refractory epilepsy still remains quite frequent condition, considered 30-40% of all patients with seizures, despite improvement in medication possibilities. Vagus Nerve Stimulator offers a neuromodulation approach when other treatment possibilities are exhausted. This minor procedure is safe but carries potential risks for anesthetic management and anesthesiologists should be aware of the physiological implications of the device and anticipate and manage possible complications.

Key Words: *anesthesia management; epilepsy; vagus nerve stimulator.*

Introduction

Epilepsy is defined as a neurological disorder manifested by an excessive and abnormal electrical neuronal discharge. It is affecting 50 million people worldwide with an incidence of 7.6 per 1,000 people (1). Refractory epilepsy still remains quite frequent condition, considered 30-40% of all patients with seizures, despite improvement in medication possibilities (1). More invasive modes of treatment include surgical resection of the epileptogenic foci or implantation of Vagus Nerve Stimulator (VNS), as the most common neuromodulation approach, when the former is not feasible (2). Careful selection of patients is mandatory, classifying patients eligible for surgical resection, deep brain stimulation or for VNS implantation (3). It is necessary to clearly explain to the patients the possible benefit of the procedure which according to many studies is seizure freedom in only 8% and 50% reduction of seizure frequency in 50-60% of the patients (1).

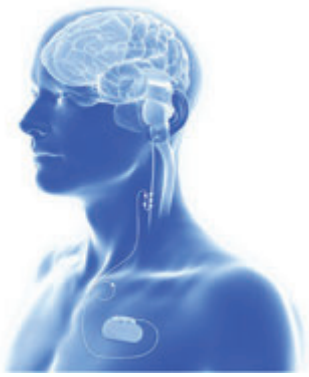


Figure 1. Schematic view of VNS components (American Journal of Neuroradiology May 2024, DOI: https://doi.org/10.3174/ajnr.A8235).

VNS System Components

Historically it was first investigated in 1938, but wasn't implanted in humans until 1988, and then needed 9 more years to be FDA approved as an adjunctive treatment for epilepsy. Its implementation is accepted in adults and children older than 4 years, but off label also in younger than one year of age (1,4). Since then, many manufacturers' modifications led to the development of the latest version of VNS. System components include a combination of stimulator (current pulse generator), single subcutaneous lead wire and platinum electrode which is wrapped around the vagus nerve with three helical coils (positive, negative and anchoring). This device can't provide any sensing of peripheral muscular or central neuronal activity, so it can't respond to current seizure activity and operates only by previously programmed parameters (5). These parameters are generally with electrical current of 1-2mA for 0.5ms and short time interval repetition of 20-30Hz for 30 seconds every 5 minutes (5,6).

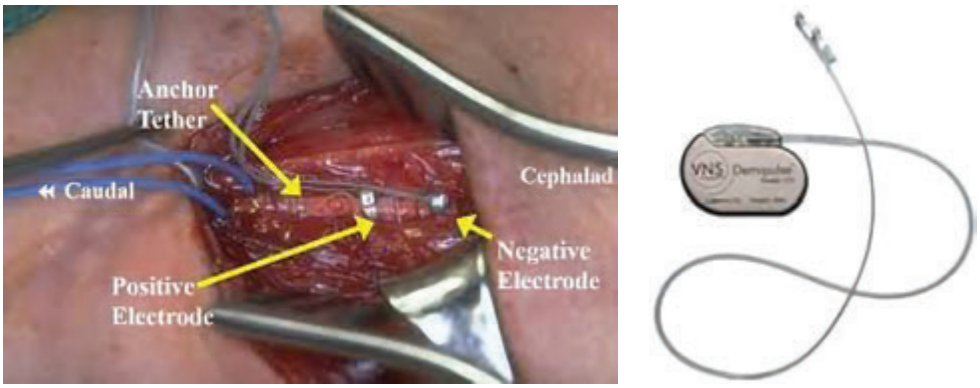


Figure 2. Intraoperative view of electrode positions and complete VNS set before implementation (https://www.cns.org/nexus/pediatric/case/vagal-nerve-stimulation-medically-intractable-epil).

VNS Surgical Implantation

After induction in anesthesia, the patient is placed in supine position with head slightly extended and turned right and elevated higher than the body. The left side of the neck and chest under the clavicle are prepared and draped. It is dissected throughout the neck layers, because the

vagus nerve is nestled between the internal jugular vein and carotid artery in the carotid sheath. Left sided vagus nerve is preferred because the sinoatrial node is innervated by the right sided vagus nerve and placing it on the right side will contribute to arrhythmia as a side effect. Even on the left vagus nerve, it is important to place it inferior to the cardiac branches so the potential cardiac effects would be minimized. Aseptic measures must be followed to prevent infections, and also careful manipulations around the nerve and large vessels so catastrophic bleeding or nerve damage be avoided. Many complications are potentially described and are divided into two groups early or related to surgery and late or related to stimulation by the device. Early complications involve perioperative arrhythmia like bradycardia, complete atrio-ventricular block or even asystole, peritracheal hematoma, hoarseness because of nerve damage, dyspnea and left vocal cord paralysis (1). Late complications usually are due to infection or poor wound healing, delayed arrhythmia, neuralgia, obstructive sleep apnea, laryngopharyngeal dysfunction and battery malfunction (1,5,6).

Anesthetic Management during VNS Implantation

The procedure is mostly performed in general anesthesia but cases using a regional technique like combination of superficial and deep cervical plexus blocks and local anesthetic infiltration in the anterior chest wall are also described. Due to changes in pharmacokinetics of anesthetics, mainly by changes in their metabolism, their doses have to be adjusted. Antiepileptic medications are cytochrome p450 enzyme inductors which contribute to faster metabolism of opioids and need for higher doses. Also, there is an up-regulation on acetylcholine receptors in the neuromuscular junction requiring also a higher dose of neuromuscular blockers to achieve satisfactory block. Due to poor control of seizures and very high risk of perioperative seizure, there is a recommendation for proceeding with the antiepileptic medications in the morning before surgery. All potential triggers that can provoke seizures like hypocarbia should be managed and avoided (5). Anesthetics like propofol and thiopental are safe to use, but drugs like ketamine are still questionable (5). There is a risk of massive bleeding due to close interconnection of the nerve with internal jugular vein and carotid artery, so blood products should be available if needed. All patients should be closely monitored because of the risk of early postoperative complications like seizures, peritracheal hematoma or vocal cord paralysis but also hemodynamic changes due to testing of the stimulator.

Mechanism of Action

Large number of studies are conducted but the exact mechanism of VNS is still debatable. Research data shows a highly complex vagal afferent network that is proposed as a modulation place for the stimulator (7). The left vagus nerve is proposed as a more favorable site for placement because it has a smaller impact on heart function, giving fibers that innervate the AV node, in comparison to the right vagus nerve that has effect on SA node in the heart. The nerve itself is quite complex and contains afferent and efferent fibers, partly myelinated A and B and partly unmyelinated C-fibers (5,7). Most of the afferent fibers terminate in Nucleus Tractus Solitarius (NTS) as a railway station for the information carried by the largest cranial nerve in the body. Then NTS, with its wide range of projections, transmits impulses to several key structures in the brainstem like the noradrenergic locus coeruleus (LC), serotoninergic raphe nucleus (RN), cerebellum, periaqueductal gray matter and parabrachial nuclei (PBN) (5,7). All these struc-

tures project to many other higher centers in the brain like the amygdala, the hypothalamus, the thalamus and limbic system (7). The data confirms that with stimulation of 1mA, alteration of neurotransmitters occurs like extracellular noradrenaline increase and also dopamine, serotonin, γ-amino butyric acid (GABA) and glycine (7).

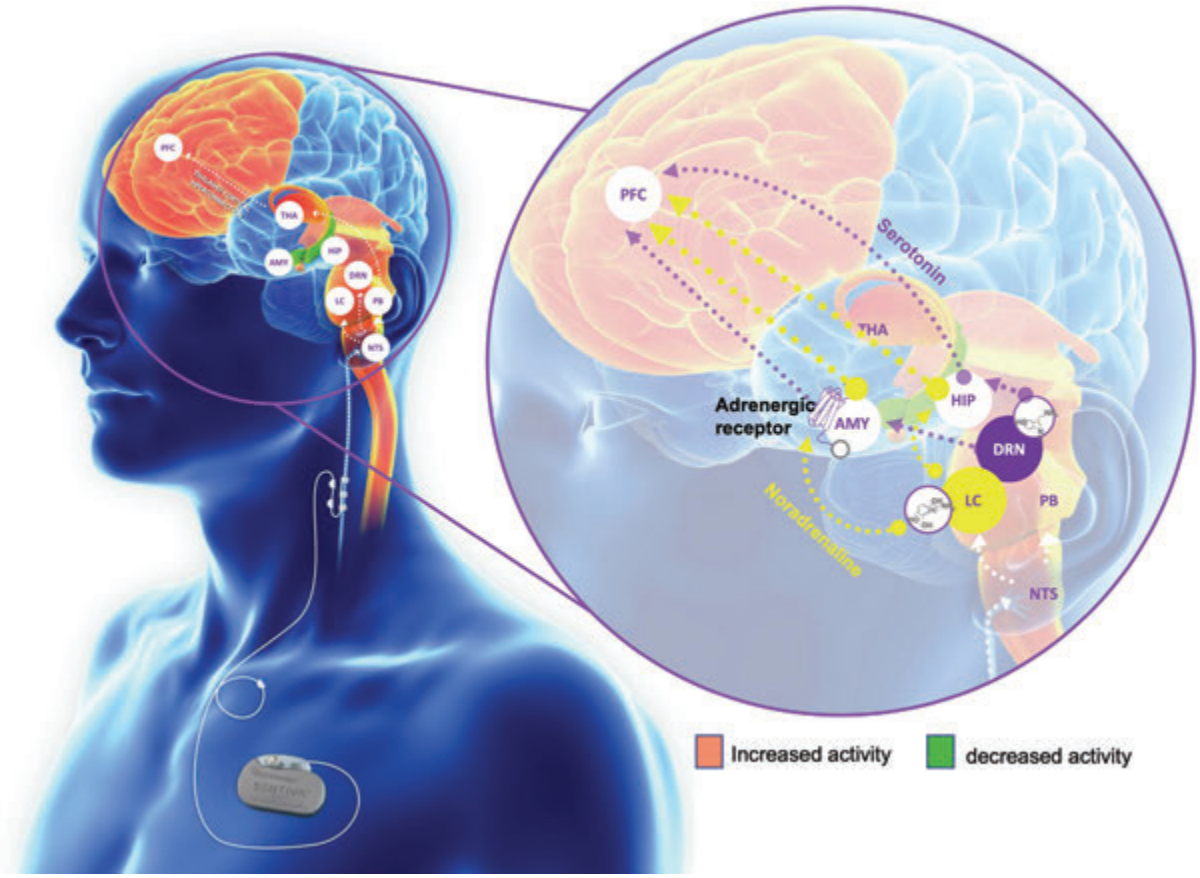
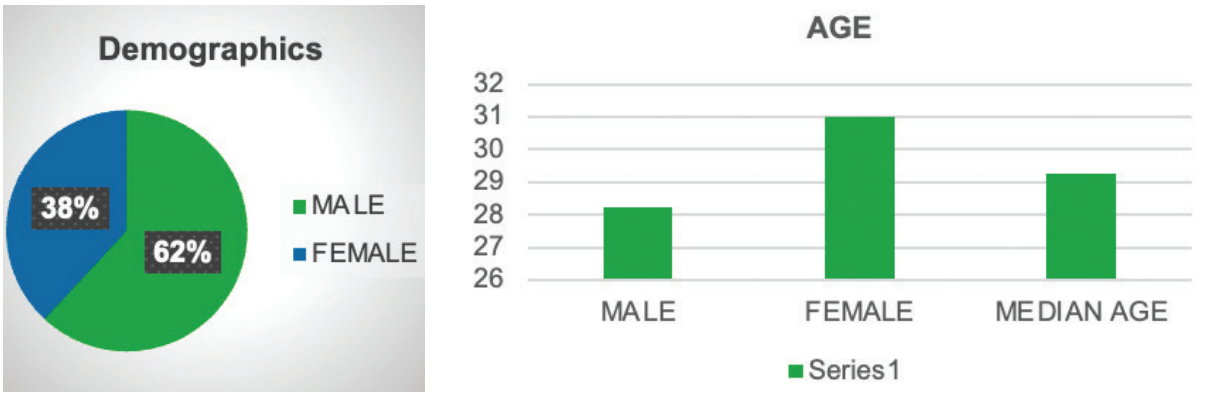


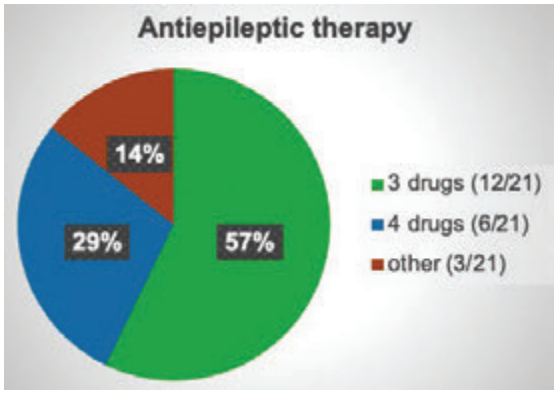
Figure 3. Afferent Vagal network and proposed structures (https://www.neuromodulationjournal.org/article/S1094-7159(22)01222-3/fulltext).

Our Center Experience

In the period from 2021 till 2025, twenty-one VNS were implanted in patients with refractory epilepsy with different etiologies and all with similar treatment with multiple antiepileptic medications in high doses. Many reasons were noted as contributors to epilepsy like trauma, tuberous sclerosis-related epilepsy, tumor resection, febrile convulsions in early childhood and Sy Dravet. Demographically 13 patients were male with median age 28 years and 8 were female with median age 31 years. More than a half of them had a need for treatment with three antiepileptics, in total 12 out of 21 patient or 57% and 6 out of 21 or 28.57% were treated with four different antiepileptics as a combined therapy. After surgery a reduction or even cessation of seizures were noted in many of the patients after a few months, but not a reduction of drug dose. Some of the isolated complications that were noted were transitory hoarseness, numbness in the left side of the face, redness of the left eye and mild hypertension noted after VNS implantation.



Graph 1. Demographic characteristics of patients.



Graph 2. Antiepileptic therapy (Patients using 3 different medications, 4 different medications and other).

Conclusion

Vagus nerve stimulation (VNS) continues to be an effective adjunctive treatment for patients who have drug-resistant epilepsy, particularly in situations where resective surgery is not an option. Despite the fact that the procedure is minimally invasive, it requires careful perioperative planning due to the potential risks associated with the anesthetic management. The experience of our center reinforces its safety profile and feasibility, with many patients experiencing a reduction in seizure burden. However, the outcomes vary, and long-term success is dependent on appropriate patient selection, clear preoperative communication, and awareness of device-related implications in future surgical or anesthetic settings. Furthermore, as the role of VNS expands, particularly into non-epilepsy indications, it is essential to take a structured and multidisciplinary approach in order to maximize its effectiveness.

References:

1. Gouveia FV, Warsi NM, Suresh H, Matin R, Ibrahim GM. Neurostimulation treatments for epilepsy: Deep brain stimulation, responsive neurostimulation and vagus nerve stimulation. Neurotherapeutics. 2024 Apr;21(3):e00308. doi: 10.1016/j.neurot.2023.e00308. Epub 2024 Jan 4. PMID: 38177025; PMCID: PMC11103217.

2. González HFJ, Yengo-Kahn A, Englot DJ. Vagus Nerve Stimulation for the Treatment of Epilepsy. Neurosurg Clin N Am. 2019 Apr;30(2):219-230. doi: 10.1016/j.nec.2018.12.005. PMID: 30898273; PMCID: PMC6432928.

3. Broderick L, Tuohy G, Solymos O,et al. Management of vagus nerve stimulation therapy in the peri-operative period: Guidelines from the Association of Anaesthetists: Guidelines from the Association of Anaesthetists. Anaesthesia. 2023 Jun;78(6):747-757. doi: 10.1111/anae.16012. Epub 2023 Apr 25. PMID: 37096456.

4. Orosz I, McCormick D, Zamponi N, et al. Vagus nerve stimulation for drug-resistant epilepsy: a European long-term study up to 24 months in 347 children. Epilepsia. 2014 Oct;55(10):1576-84. doi: 10.1111/epi.12762. Epub 2014 Sep 17. PMID: 25231724.

5. Hatton KW, McLarney JT, Pittman T, Fahy BG. Vagal nerve stimulation: overview and implications for anesthesiologists. Anesth Analg. 2006 Nov;103(5):1241-9.doi: 10.1213/01.ane.0000244532.71743.c6. PMID: 17056962.

6. Giordano F, Zicca A, Barba C, Guerrini R, Genitori L. Vagus nerve stimulation: Surgical technique of implantation and revision and related morbidity. Epilepsia. 2017 Apr;58 Suppl 1:85-90. doi: 10.1111/epi.13678. PMID: 28386925.

7. Carron R., Roncon P., Lagarde S., Dibué M., Zanello M., Bartolomei F. 2023. Latest Views on the Mechanisms of Action of Surgically Implanted Cervical Vagal Nerve Stimulation in Epilepsy.Neuromodulation 2023; 26: 498–506.

8. Shetty A, Pardeshi S, Shah VM, Kulkarni A. Anesthesia considerations in epilepsy surgery. Int J Surg. 2016 Dec;36(Pt B):454-459. doi: 10.1016/j.ijsu.2015.07.006. Epub 2015 Jul 15. PMID: 26188082.

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MASS CASUALTY EVENT PREPAREDNESS AND ANESTHESIA MANAGEMENT IN THE EMERGENCY DEPARTMENT AND INTENSIVE CARE UNIT

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Abstract

Mass casualty events whether natural disasters, terrorist attacks, pandemics or large-scale accidents overwhelm healthcare systems, emergency departments and ICUs in minutes. High acuity situations require rapid coordination, strategic resource allocation and seamless inter-disciplinary collaboration. Anesthesiologists are uniquely positioned to play a central role in all phases of the response due to their expertise in airway management, hemodynamic stabilization, pharmacologic sedation and critical care. Their role extends beyond the OR to initial triage, emergency procedural support and ongoing management of ventilated and critically ill patients in the ICU. This article reviews current evidence and operational best practices to examine the role of anesthesiology in mass casualty events preparedness and response. Key areas of focus include disaster planning, ventilator triage, crisis standards of care, sedation protocols and ICU surge capacity. By highlighting the clinical, operational and ethical aspects of anesthesia management in mass casualty events, this article synthesizes current literature and best practices, emphasizing the anesthesiologists' role in preparedness, immediate response and postoperative care during mass casualty events.

Key Words: *critical care, disaster preparedness, mass casualty events, triage protocols.*

Introduction

Mass casualty events present a challenge to the healthcare system requiring a coordinated, multidisciplinary response to optimize patients' outcomes (1). As medical professionals, particularly anesthesiologists and critical care physicians, our role in the ER and ICU is pivotal in stabilizing and managing critically injured patients. These events arising from natural disasters, terrorist incidents, industrial catastrophesor pandemics necessitate meticulous preparedness and strategic anesthesia management to mitigate morbidity and mortality effectively (2).

Mass casualty events are defined as incidents in which the number and severity of casualties exceed the immediate capabilities of the local healthcare infrastructure (3). In the current era characterized by increasing geopolitical instability, climate change-related natural disaster and global pandemics, these events have become more frequent and complex. They necessitate a rapid and highly coordinated medical response (4). Hospitals, particularly tertiary care centers, must be prepared to receive and manage a sudden influx of critically ill or injured patients (5).

Anesthesiologists associated with perioperative care are playing critical roles beyond the confines of the operating theater. Their skill set uniquely qualifies them to manage airways, provide procedural sedation, conduct resuscitation and oversee critical care interventions under conditions of uncertainty and resources scarcity, from establishing emergent airways in chaotic emergency room settings to leading intensive care units overwhelmed with patients requiring mechanical ventilation and vasopressor support (6). In particular, the COVID-19 pandemic acted as a global mass casualty event revealing critical gaps in hospital preparedness and highlighting the role of anesthesiologists. During this time many wereperforming intubations and managing ventilators in unfamiliar and often high-risk environments. This unprecedented crisis underscored the need for a more integrated and systematic approach to anesthesia involvement in disaster response (7). Disaster preparedness is not solely about having equipment or protocols in place, it involves interdisciplinary collaboration, simulation-based training, real-time communication pathways and psychological readiness (8). Anesthesiology departments must be actively engaged in hospital disaster committees and scenario planning. As ICU capacities are rapidly overwhelmed by the influx of severely injured or unstable patients, anesthesiologists are tasked with delivering comprehensive care in high-pressure, resource-constrained environments (9).

Clinical Management during Mass Casualty Events

Mass casualty events are defined as large-scale incidents in which the number of injured overwhelms the capacity and resources of a local healthcare system. These scenarios are increasingly frequent due to geopolitical instability, climate-related disasters, pandemics and industrial accidents. The medical response to such events requires a well-orchestrated, interdisciplinary and adaptable approach that integrates emergency medicine, trauma surgery, anesthesia, nursing and critical care. This essay explores the elements of emergency and critical care during mass casualty events, triage, resuscitation, critical care strategies and the operational adjustments needed to sustain effective healthcare delivery under extreme situations.

Triage and Early Response

Triage is the foundation for efficient care delivery during a mass casualty event. The primary goal is to prioritize care based on injury severity, prognosis and available resources (10). Two of the most widely accepted systems are START (Simple Triage and Rapid Treatment) and SALT (Sort, Assess, Lifesaving Interventions, Treatment/Transport) (Figure 1, 2). These models stratify patients into categories such as Immediate, Delayed, Minor and Expectant (11).The Expectant category is ethically complex and acknowledges the need to allocate limited life-saving interventions where they are the most likely to succeed (12).

Initial triage occurs at the disaster site or at the emergency department entrance, often in chaotic conditions. Rapid assessment includes airway, breathing, circulation, disability (neurologic status) and exposure. Healthcare teams must be trained to make fast high-stakes decisions that can directly influence survival. Triage officers are typically experienced clinicians trained in disaster medicine or emergency care and their judgments must be supported by standardized protocols (13).

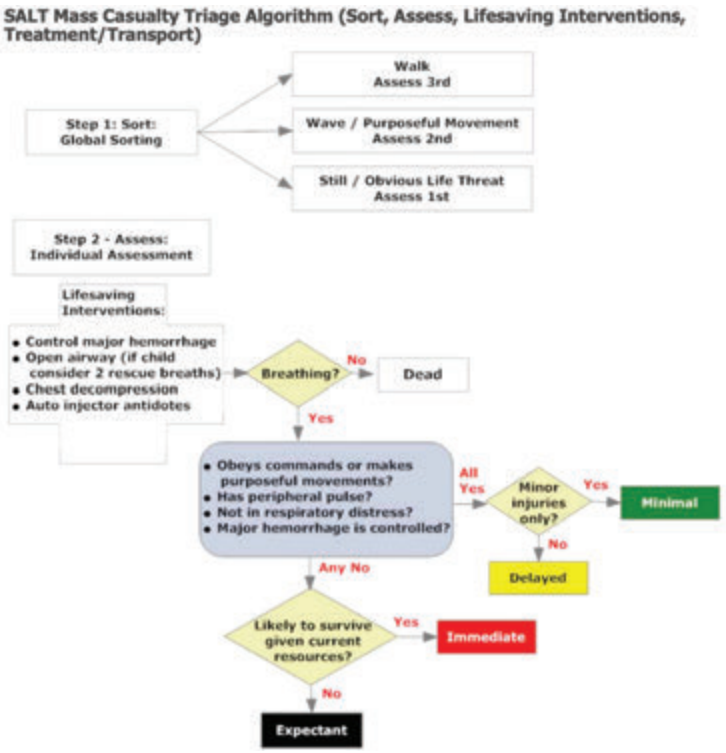

Figure 1: Triage Decision-MakingProcess (14)

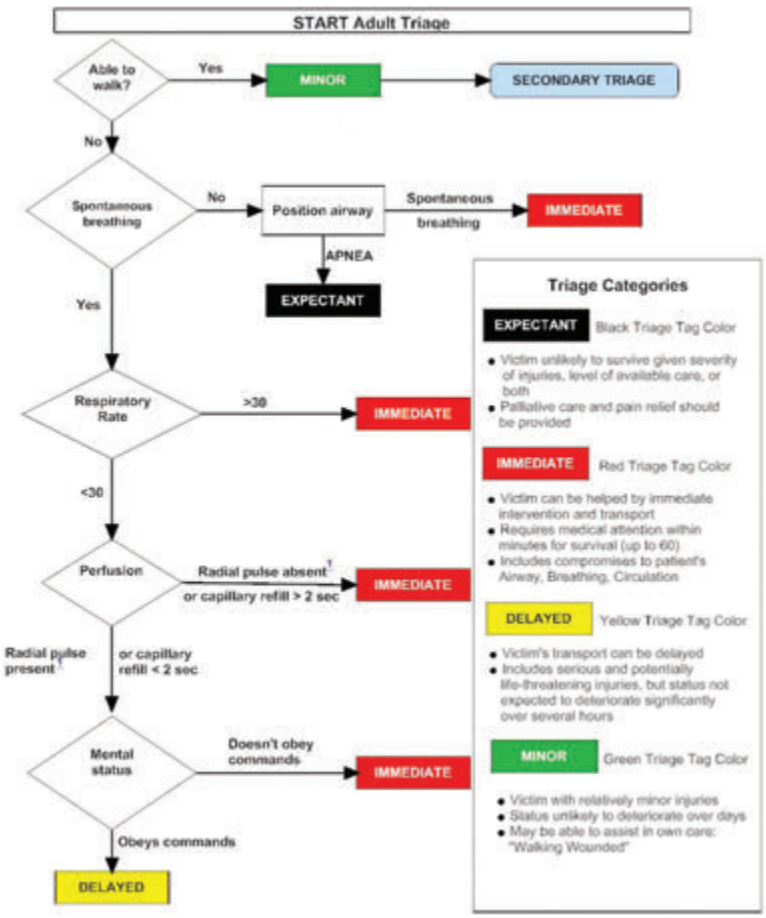

Figure 2: Triage Decision-Making Process (14)

Ethical Decision Making in Triage

Ethical issues did come into consideration during MCE triage situations where scarce resource limitation necessitates hard decisions (15). The Expectant category positions a patient as a victim unlikely to survive given available resources-evidence of one of those difficult decisions in triage. Again, guidelines from Disaster Ethics Protocols set down in past global crises can be used to administer such techniques in an ethical manner (16). Ethical frameworks were thus put in place for allocating scarce resources, such as ventilators, in the 2020 First Wave of COVID-19. Likewise, the events in 2020 from the Beirut blast threw into question the presence of clear triage protocols that allow for ethical management of the overburdened influx of patients. These principles may, for instance, include maximizing benefits, treating people equally, giving priority to instrumental value and giving priority to the worst off (16).

Airway and Respiratory Management

Airway compromise is a leading cause of preventable mortality in trauma and disaster settings. Early airway intervention is critical and must be executed with both speed and precision (figure 3). Rapid Sequence Intubation (RSI) remains the preferred method for securing airways in patients with depressed consciousness, facial trauma or respiratory failure (17). Induction agents such as ketamine (due to its sympathetic stimulation) or etomidate (for hemodynamic stability and neuroprotection) are commonly utilized in conjunction with neuromuscular blockers like succinylcholine or rocuronium. In environments where intubation is unsuccessful or not immediately possible, supraglottic airway devices (laryngeal mask airways) offer effective temporizing measures. Inhalational injuries, aspirationand thoracic trauma can complicate airway management and require multidisciplinary inputincluding respiratory therapy and pulmonology (18). Mechanical ventilation, once established, must adhere to lung-protective principles especially in patients with acute respiratory distress syndrome (ARDS), a common complication in mass casualty events involving inhalation burns, sepsis, or blunt chest trauma. Low tidal volume ventilation (6mL/kg ideal body weight), appropriate positive end-expiratory pressure (PEEP) and plateau pressure monitoring help to prevent ventilator-induced lung injury.

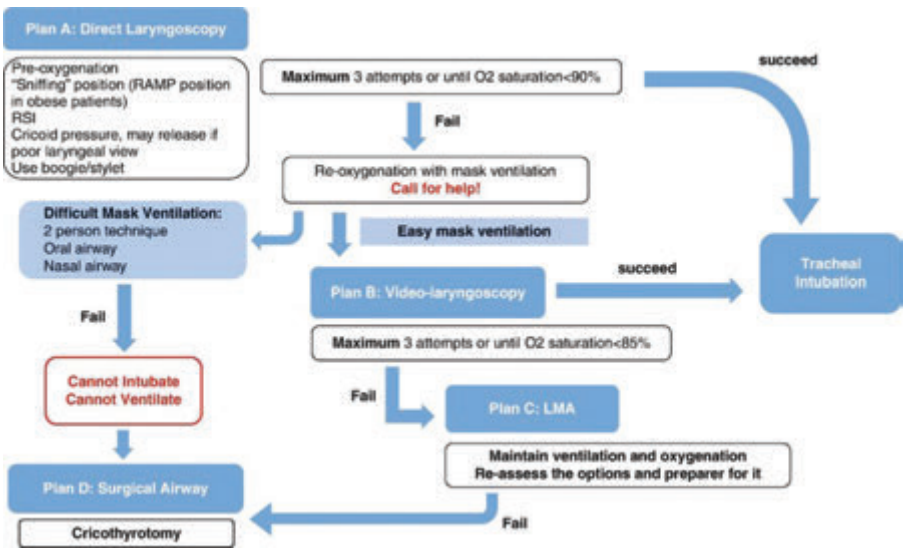

Figure 3: Algorithm Airway Management in Mass Casualty Events (19)

Hemodynamic Resuscitation and Shock Management

Circulatory collapse from hemorrhagic shock, sepsis or cardiac trauma is another critical threat in mass casualty events patients. Damage control surgery is for patients with massive trauma or exsanguination focused on resuscitation and minimizing anesthesia time. The goal of damage control surgery is to control bleeding and contamination deferring definitive repair until the patient is stabilized (20). Damage control resuscitation is early hemorrhage control, permissive hypotension (in absence of traumatic brain injury) and balanced transfusion of blood products in a 1:1:1 ratio of red blood cells, plasma and platelets (21). This strategy aims to mitigate the "lethal triad" of coagulopathy, acidosis and hypothermia that drives poor trauma outcomes. In patients with traumatic brain injuries maintaining adequate cerebral perfusion pressure, minimizing intracranial pressure and avoiding hypoxia and hypercapnia are key anesthetic objectives. Sedation, controlled ventilation and osmotic agents are needed. Fluid resuscitation must be carefully managed to avoid exacerbating edema, compartment syndromes or dilutional coagulopathy. Balanced crystalloids such as Lactated Ringer are preferred over normal saline to reduce the risk of hyperchloremic acidosis. Vasopressors like norepinephrine and vasopressin may be necessary in patients with persistent hypotension despite adequate volume resuscitation. Invasive hemodynamic monitoring through arterial lines and central venous catheters, as well as the use of point-of-care ultrasound, assists in real-time decision-making regarding fluid responsiveness and cardiac function.

Critical Care and ICU Expansion

As the acute resuscitation phase transitions to critical carethe focus shifts to organ support, prevention of secondary complicationsand eventual recovery. Intensive care units often reach capacity quickly during mass casualty events. Therefore, hospitals must have pre-planned surge strategies that allow perioperative areas such as post anesthesia care units, procedural suites and even operating rooms to be converted into intensive care units (22). Patients in the ICU frequently require prolonged mechanical ventilation, renal support, vasopressor therapy and complex nutritional management. In patients unresponsive to fluid resuscitation, vasopressors such as norepinephrine (first line) and vasopressin (as adjunct) are used to maintain perfusion pressure and prevent organ failure. Continuous renal replacement therapy is frequently needed in patients with acute kidney injury secondary to rhabdomyolysis, hypoperfusion or sepsis. For those with refractory hypoxemia or cardiac collapse, extracorporeal membrane oxygenation (ECMO) may be considered, although it is resource-intensive and requires highly specialized personnel. Neurocritical care is a parallel priority in patients with traumatic brain injuries, spinal cord injuries, or anoxic brain damage. The maintenance of cerebral perfusion pressure, prevention of intracranial hypertension and the judicious use of osmotic therapy are key components. Sedation protocols in the ICU are adapted to preserve neurologic examination and prevent delirium. Agents such as dexmedetomidine offer anxiolysis and light sedation without respiratory depression, while propofol and midazolam are reserved for deeper sedation when indicated.

Pain Control and Sedation

Pain and agitation in critically ill patients can lead to increased metabolic demand, sympathetic overdrive and worsened outcomes. Acute pain leads to elevated catecholamine levels, immuno-

suppressionand impaired ventilation, all of which can worsen outcomes. A multimodal analgesic strategy is essential to reduce opioid consumption and minimize side effects. This includes the use of acetaminophen, NSAIDs (if renal function is intact), local anesthetics and regional techniques such as nerve blocks or epidurals when feasible.Sedation must be carefully titrated to balance comfort with the ability to conduct neurologic assessments. During mass casualty events the scarcity of agents may necessitate prioritization of long-acting or easily stored drugs. Providers must also remain vigilant for the development of ICUacquired delirium, necessitating regular sedation interruptions and cognitive assessments.

Preparedness, Training and Systems-Based Readiness

Effective disaster response hinges not just on individual competence but on institutional readiness. Hospitals must adopt an Incident Command System to establish a structured chain of commands, promote interagency collaboration and streamline communication. Pre-event planning should include hospital-wide simulation exercises that incorporate mass triage, patient flow management and mock casualties (23).

Clinical teams must receive ongoing education in trauma care, critical care and disaster ethics. Simulation-based training is particularly valuable, enabling teams to rehearse rare but high-impact scenarios in a controlled setting. Such training improves team coordination, leadership under pressure, and adherence to best practices (24).

Mass casualty events represent a test of a healthcare system's resilience, preparedness and cohesion. Success in managing such events requires a well synchronized response that integrates rapid triage, advanced critical care, ethical resource allocation and continual training. While clinical skills are vital, it must be coupled with systems-level planning, real-time communication and flexibility in repurposing resources (24). Whether the casualties are from an earthquake, explosion or pandemic, the guiding principles remain constant: preserve life, alleviate suffering and maintain the integrity of the health system under duress. In such moments, the entire healthcare team like emergency physicians, surgeons, intensivists, anesthesiologists, nurses and support staffbecomes a single organism functioning with one purpose, to restore order to chaos.

Discussion

In mass casualty events, taking care of seriously injured or critically ill patients is a huge challenge. Anesthesiologists play a key role in this response, both in the emergency room and in the intensive care unit (25).Their responsibilities range from rapid intubation and resuscitation to the provision of sedation, analgesia and long-term mechanical ventilation. These responsibilities are often carried out under pressure with limited resources and incomplete information, and experience and preparedness are essential for patients' outcomes(26). In the emergency room anesthesiologists are frequently called upon to assist with airway management, hemodynamic stabilization and procedural sedation for trauma patients and critically ill patients. Their expertise in pharmacologic agents allows for efficient and safe interventions in unstable patients. Moreover, the anesthesiologists' ability to work in high-stress environments, make quick decisions, and function in interdisciplinary teams makes them invaluable during the initial phases of disaster responses. In the ICU, anesthesiologistsplay a central role in managing ventilation

strategies, sedative regimens, fluid resuscitation and vasopressor support. During mass casualty events ICU often exceed capacity requiring non-traditional spaces such as postanesthesia care units or operating rooms to be repurposed. Anesthesiologists are uniquely qualified fortheir ability to manage multiple critically ill patients simultaneously.

An overlooked aspect of anesthesiology involvement is participation in the hospital disaster planning and preparedness activities.Anesthesiologists should be involved in emergency planning and taking part in drills that simulate real-life crises with difficult decisions when resources are depleted.Ethical considerations such as allocation of resources pose serious moral dilemmas. In these situations, anesthesiologists may be asked to participate in or even lead triage teams that determine who receive potentially life-saving interventions (27). Having established guidelines with ethical principles and support by institutional leadership can help mitigate the moral burden on clinicians.This can help reduce the emotional and moral stress on healthcare workers making hard decisions in the middle of a disaster (28).

Conclusion

Mass casualty preparedness and anesthesia management demand a systematic, well-coordinatedand multidisciplinary approach to optimize patients' survival, reduce morbidity and ensure the effective utilization of scarce resources. In such highacuity and resource limited scenarios, anesthesiologists and intensivists serve as essential frontline providers. Their expertise in airway management, resuscitation, perioperative care, critical care pharmacology and mechanical ventilation uniquely equips them to manage both the acute and prolonged phases of patient care in the emergency room and intensive care unit. Beyond their clinical skillsets, anesthesiologists also contribute meaningfully to disaster planning, ethical decisionmaking under crisis standards of care and systems level problem solving. Their role in simulation training, triage development and rapid response protocol implementation enhances institutional resilience and preparedness.An effective mass casualty response cannot succeed without strong interdisciplinary collaboration, frequent drills, logistical readiness and a clear delineation of roles. Integrating anesthesiology more deeply into institutional emergency protocols, command structures and preparedness committees ensures not only better outcomes during disasters but also a more adaptive and unified healthcare response.

References:

1. Kelen GD, McCarthy ML. The science of surge. Acad Emerg Med. 2006;13(11):1089–1094.
2. Christian MD, Sprung CL, King MA, et al. Triage: care of the critically ill and injured during pandemics and disasters: CHEST consensus statement. Chest. 2014;146(4):e61S–e74S.
3. Hick JL, Hanfling D, Wynia MK, Pavia AT. Duty to plan: health care, crisis standards of care, and novel coronavirus SARS-CoV-2. NAM Perspectives. 2020.
4. Ciottone GR. Disaster Medicine. 2nd ed. Elsevier; 2015.
5. Devereaux AV, Dichter JR, Christian MD, et al. Definitive care for the critically ill during a disaster: a framework. Chest. 2008;133(5):1115–1123.

6. Matava CT, Kovatsis PG, Lee JK, et al. Pediatric airway management in COVID-19 patients. AnesthAnalg. 2020;131(1):61–73.
7. Uppal V, Sondekoppam RV, Landau R, El-Boghdadly K, Narouze S. Neuraxial anaesthesia and peripheral nerve blocks during the COVID-19 pandemic. Reg Anesth Pain Med. 2020;45(7):581–588.
8. Sprung CL, Zimmerman JL, Christian MD, et al. Recommendations for intensive care unit and hospital preparations for an influenza epidemic or mass disaster. Intensive Care Med. 2010;36(3):428–443.
9. Thomas MJ, Sanford P. Operating rooms and PACUs as temporary ICUs. Anesthesiol Clin. 2020;38(4):785–794.
10. del Carmen Cardós-Alonso, M., Otero-Varela, L., Redondo, M. *et al*. Extended reality training for mass casualty incidents: a systematic review on effectiveness and experience of medical first responders. *Int J Emerg Med* **17**, 99 (2024).
11. Lerner EB, Schwartz RB, Coule PL, et al. Mass casualty triage: an evaluation of the data and development of a proposed national guideline. Disaster Med Public Health Prep. 2008;2(S1):S25–S34.
12. Persad G, Wertheimer A, Emanuel EJ. Principles for allocation of scarce medical interventions. Lancet. 2009;373(9661):423–431.
13. Iserson KV. Triage in medicine, part I: concept, history, and types. Ann Emerg Med. 1992;19(3):268–272.
14. Crisis Medicine. START, SALT, and RAMP: Triage in a Mass Casualty Event. Crisis Medicine. Published October 4, 2022. Accessed May 14, 2025.https://www.crisis-medicine.com/start-salt-and-ramp-triage-in-a-mass-casualty-event/
15. Cuthbertson J, Penney G. Ethical Decision Making in Disaster and Emergency Management: A Systematic Review of the Literature. *Prehospital and Disaster Medicine*. 2023;38(5):622-627. doi:10.1017/S1049023X23006325.
16. Ekmekci, P.E., Zhang, L. and Crawley, F.P. (2025) 'Ethics Underpinning Data Policy in Crisis Situations', Data Science Journal, (24), p. 4.
17. Sakles JC, Laurin EG, Rantapaa AA, Panacek EA. Airway management in the emergency department. Emerg Med Clin North Am. 1998;16(1):27–48.
18. Cook TM, Woodall N, Frerk C. Major complications of airway management in the UK. Br J Anaesth. 2011;106(5):617–631.
19. Barak, M., Leiser, Y., Kluger, Y. (2019). Airway Management in Trauma Patients. In: Aseni, P., De Carlis, L., Mazzola, A., Grande, A.M. (eds) Operative Techniques and Recent Advances in Acute Care and Emergency Surgery. Springer, Cham.
20. Rotondo MF, Schwab CW, McGonigal MD, et al. Damage control: an approach for improved survival in exsanguinating penetrating abdominal injury. J Trauma. 1993;35(3):375–382.
21. Holcomb JB, del Junco DJ, Fox EE, et al. The Prospective, Observational, Multicenter, Major Trauma Transfusion (PROMMTT) Study. JAMA Surg. 2013;148(2):127–136.
22. Curtis JR, Rubenfeld GD. Managing ICU triage during a pandemic crisis: the importance of process. Crit Care Med. 2020;38(6 Suppl):S1–S3.

23. Gaba DM. Crisis resource management and teamwork training in anesthesia. Br J Anaesth. 2010;105(1):3–6.

24. Ramaswamy V, Li J, Munoz R, et al. Simulation-based disaster preparedness training improves hospital staff's response performance. Disaster Med Public Health Prep. 2021;15(3):353–359.

25. Wax RS, Christian MD. Practical recommendations for critical care and anesthesiology teams caring for novel coronavirus patients. Can J Anaesth. 2020;67(5):568–576.

26. Rosenbaum L. Facing COVID-19 in Italy—ethics, logistics, and therapeutics on the epidemic's front line. N Engl J Med. 2020;382(20):1873–1875.

27. Emanuel EJ, Persad G, Upshur R, et al. Fair allocation of scarce medical resources in the time of COVID-19. N Engl J Med. 2020;382(21):2049–2055.

28. Truog RD, Mitchell C, Daley GQ. The toughest triage—allocating ventilators in a pandemic. N Engl J Med. 2020;382(21):1973–1975.

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INHALATIONAL ANESTHETICS WITH THEIR PHARMACOKINETIC PROPERTIES

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Abstract

Inhalational anesthetics are the most common drugs used for providing general anesthesia for surgery. They are used to induce unconsciousness, amnesia and immobility. In search for ideal anesthetic gas there have been discovered many agents, but sadly the perfect inhalational drug has not been produced. The pharmacokinetics of inhalational anesthetics depends on their physical properties. The rate of uptake and elimination of the inhalational anesthetics from the alveoli in mainly depends on their blood solubility. The main driving force of absorption and distribution of the gas through the body is partial pressure gradient on both sides of each barrier in the gas flow, and the therapeutic effect of the gas depends of the partial pressure of the anesthetic in the brain. All inhalational anesthetics, with exception of nitrous oxide and xenon, are metabolized (in different degrees) in liver via cytochrome P450 enzymes. The potency of different inhalational anesthetics is expressed via minimum alveolar concentration, which is defined as concentration of the anesthetics that prevent movement in 50% of the patients in response to surgical incision under standard conditions. Nitrous oxide and volatile halogenated ethers (desflurane, isoflurane, sevoflurane) are examples of medical gases that are greenhouse gases with great impact to global warming. This article summarizes a brief historical timeline of the inhalational anesthetics, mechanisms of action, physical characteristics and pharmacokinetic properties of inhalational agents with a short overview of their toxicity and pollution of the environment.

Keywords: *F_A/F_I ratio, greenhouse gas, inhalational anesthetics, minimum alveolar concentration, pharmacokinetics, solubility.*

Historical Prospective

Inhalational anesthetics were discovered way before the induction of intravenous anesthetic drugs. Since 1840's, there has been continuous search for ideal gas. The discovery of inhalational agents with some of their characteristics is presented in the following timeline:

- In **1842**, dentist Horace Wells for the first time used **nitrous oxide** on himself for pain-relief. Two years later, he publicly demonstrated painless dental surgery using nitrous oxide which was not completely successful, and he was discredited. Nitrous oxide is colorless gas with sweet odor and taste, but also weakest general anesthetic.
- In 1842, Crawford Long administered **diethyl ether** to a patient (1). Four years later, on October 16th, **1846**, Boston dentist William Morton publicly demonstrated ether's anes-

thetic properties. This day is now commemorated as "Ether Day". Diethyl ether is a highly flammable colorless volatile liquid with hash side effects like nausea and vomiting.

- **Chloroform** was introduced in **1847** by obstetrician from Edinburgh, James Simpson, as nonexplosive alternative to ether. It is colorless with sweet smelling, dense liquor, but due to several unexplained intraoperative deaths and numerous cases of hepatotoxicity, chloroform was stopped for usage.
- The modern era of volatile anesthetics began with the discovery of **halothane** in **1951** by Suckling, and in **1956** it was introduced into clinical practice. The same year when halothane was discovered, **xenon** was for the first time used as a surgical anesthetic by American anesthesiologist Stuart C. Cullen (2). Halothane is colorless, potent volatile anesthetic with sweet smell and unstable on light. Due to unpredictable liver damage and his dysrhythmogenic effects on myocardium, the search for better volatile anesthetic continued.
- **Methoxyflurane** was introduced in **1960** into clinical practice. It is a colorless liquid with a fruity odor. Although, it is extremely potent, it has slow onset and offset times. Few years after its introduction, dose-related nephrotoxicity was confirmed with methoxyflurane anesthesia.
- **In 1973, enflurane** was introduced in clinical practice. It has the same characteristics as halothane (colorless liquid with sweet odor, sensitive on light and fast induction) but without its side effects. Enflurane can cause dose-related seizures.
- **Isoflurane** was introduced into clinical practice in **1981**, as a colorless, nonflammable liquid with pungent odor.
- **In 1992 desflurane** was discovered and two years later**, sevoflurane.** Desflurane is a colorless, nonflammable liquid with a pungent odor. It has fast induction, low potency but is expensive. Sevoflurane is a colorless, nonflammable liquid with pleasant odor. Its induction is slower than with desflurane.

Mechanism of Action

All volatile anesthetics have the same mechanism of action, but the exact mechanism is still unknown. First, Meyer (1899) and Overton (1901) developed a theory in which they believed that anesthetics bind to the bilayer lipid membrane and that their potency correlates to their solubility in lipids (3). Their theory explains that the anesthetic agent molecules bond to target sites on the lipid layer after an anesthetic agent reaches a critical level in a lipid layer. This process causes dissolution of the lipid layer of the brain cells, and the brain reaches an anesthetized state or unconscious. Later in the 1970's, researchers demonstrated that anesthetics did not need lipid target sites for binding and that the primary site of action for anesthetics, including the inhalational anesthetics, involve proteins (4). At the end of the last century, many studies showed that the main target for inhaled anesthetics most likely are ligand gated ion channels proteins (GABA receptors, glycine, nicotinic acetylcholine, NMDA) (5-7). Physiological function of GABA and glycine receptors is to inhibit the postsynaptic excitation, so volatile anesthetics sensitized these receptors and prolong the inhibition (8, 9). Inhaled anesthetics not only have postsynaptic effect, but they also have presynaptic effect by blocking Na+ channels (NMDA receptors) and inhibited the presynaptic excitatory neurotransmitter release (10) (Figure 1). Amnesia occurs

probable due to impact of inhalational anesthetics to nicotinic acetylcholine receptors (11).

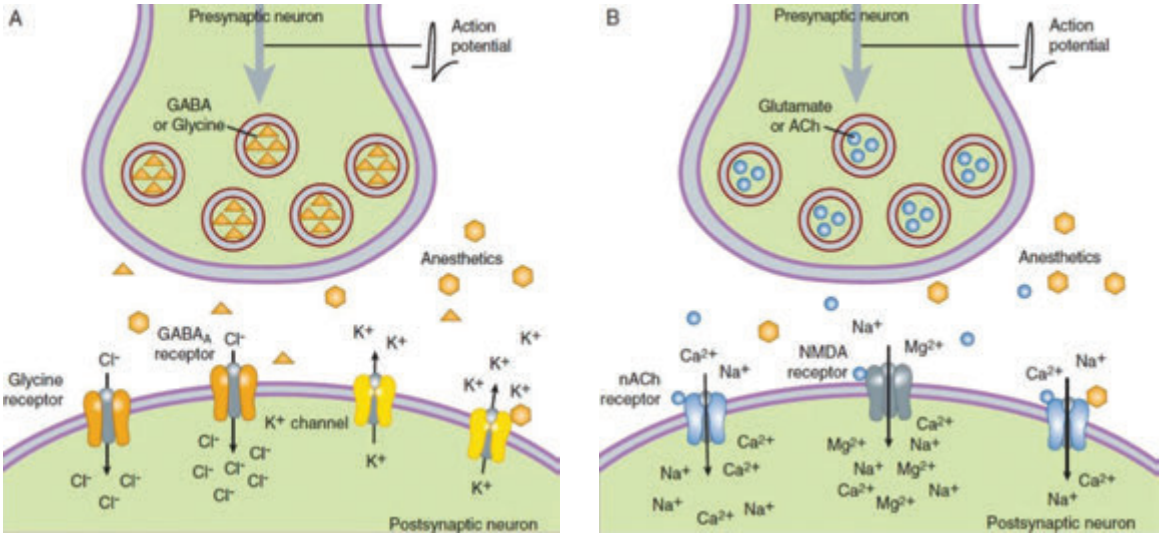

Figure 1. Mechanism of action of inhal→→→ational anesthetics. A – inhibitory synapse: Inhalational anestetic enhence the binding of GABA and glycine for the $GABA_A$ receptor and increase Cl^- influx causing hyperpolarized cell difficult to depolarize (↓ excitability). B – excitatory synapse: Inhalational anesthetics block Na^+ channels (NMDA receptors) and inhibit the presynaptic excitatory neurotransmitter release (12).

GABA receptors have an inhibitory role in the adult brain, whereas, in growing developing brain GABA receptors are the main excitatory neurotransmitters. In a young child's brain, GABA receptor opens calcium channels and increases calcium influx in the cell causing cell apoptosis (see Figure 1B). There is concern of using inhalational anesthetics in the youngest patients because, due to their exposure to inhalational anesthesia, it can cause a lasting deficit in behavior, learning and memory (13).

Physical Properties

The main physical characteristics of the inhalational anesthetics are shown in Table 1.

Table 1. Physical characteristics of inhalational anesthetics.

	Halothane	Enflurane	Isoflurane	Sevoflurane	Desflurane	N_2O	Xenon
Boiling point at 1 atm	50.2	56.5	48.5	58.5	22.8	-88.5	-108.1
Vapor pressure at 20°C	243	172	240	160	669	39000	-
MAC in 30-60 years, at 37°C	0.75	1.7	1.2	2	6	104	60-70
Blood: gas solubility at 37°C	2.5	1.8	1.4	0.65	0.45	0.47	0.14
Brain: blood solubility	1.9	1.4	1.6	1.7	1.3	1.1	
Fat: blood solubility	51.1	36	44.9	47.5	27.2	2.3	

	Halothane	Enflurane	Isoflurane	Sevoflurane	Desflurane	N_2O	Xenon
Muscle: blood solubility	3.4	1.7	2.9	3.1	2.0	1.2	
Recovered as metabolites (%)	20-40	2.4	0.2	2-5	0.02		
Metabolites	TFA*		TFA*	HFIP*	TFA*	-	-
Preservative	Thymol	No	No	No	No		
Stable in moist CO2 absorber	No	Yes	Yes	No	Yes		

*Trifluoroacetate (TFA), Hexafluoroisopropanol (HFIP)

Vapor pressure is the partial pressure exerted by the vapor in equilibrium with its liquid phase. On this pressure, equal parts of the liquid phase evaporate into gaseous phase and equal parts of gas condensate into liquor.

The boiling point is the temperature at which the liquor turns into gas or the temperature at which the vapor pressure equals to the surrounding atmospheric pressure. If the atmospheric pressure is low, like at higher altitudes, the boiling point **decreases** because there's less pressure holding the liquid together. Desflurane's boiling point of 23.5°C is near to room temperatures. It is stored in a special container (under vapor pressure) in order to escape boiling at room temperature.

According to *Dalton's law*, total pressure in mixture of gases is equal to the sum of the partial pressures of each individual gas in the mixture. Mathematically, it's expressed as:

$$P_{total}=P_1+P_2+P_3+\cdots$$

where P_{total} is the total pressure of the gas mixture and P_1, P_2, P_3 are the partial pressures of each gas. Each gas in a mixture acts **independently**, which means that partial pressure of one gas is the pressure it would exert alone in the container at the same temperature and volume. Inhalational anesthetic partial pressures are expressed as volume percent (vol%) indicating the percent of the total volume contributed by a specific gas.

The partial pressure of a gas in solution refers to the pressure that the gas **would exert** if it were alone in a container at the same temperature. *Henry's law* describes how gases dissolve in liquids based on their **partial pressure**. According to this law, the concentration of a gas in a liquid is **directly proportional** to the partial pressure of that gas above the liquid. The higher the pressure, the more gas dissolves into the liquid.

$$C = k \times P$$

where C is concentration of the gas in the liquid (mol/L), k is Henry's law constant (solubility constant specific to each gas-liquid pair), and P is partial pressure of the gas above the liquid (atm).

It is important to talk of partial pressures, because gases equilibrate based on partial pressures, not on concentrations. Inspired concentration or fractional concentration of inspired anesthetic is used as terminology rather than partial pressure.

$F = P_{anesthetic}/P_{barometric}$

where F is fractional concentration of anesthetic, $P_{anesthetic}$ is partial pressure of anesthetic. This equation shows that the fractional concentration of anesthetic is directly proportional to its partial pressure.

Pharmacokinetics of Inhalational Anesthetics

Pharmacokinetics studies how the body reacts to an administered drug during the entire time while is exposed to it. Pharmacokinetics of the inhalational agent focuses on four processes:

1. Absorption or uptake (wash in) – process where the inhaled volatile agent is transported from the lung to the bloodstream,
2. Distribution or tissue uptake – process where the inhaled volatile agent is transported from the bloodstream to the tissue (mainly to the effective tissue or the brain),
3. Metabolism – process where the inhaled agent breaks down in the body, often in the liver, into substances that can be more easily eliminated,
4. Elimination or wash out – process where the inhaled agent and its metabolites are removed from the body, through the lungs or through urine and feces.

The main driving force of absorption and distribution of the gas through the body is partial pressure gradient on both sides of each barrier in the gas flow, and the therapeutic effect of the gas depends of the partial pressure of the anesthetic in the brain.

P_A (alveolar partial pressure) ↔ P_a (arterial partial pressure) ↔ P_{brain} (brain partial pressure)

Equilibration of the partial pressures of alveolar and inspired agent is also known as "Wash in" (F_A/F_I) and this rate can rise from 0 to 1 (Figure 2).

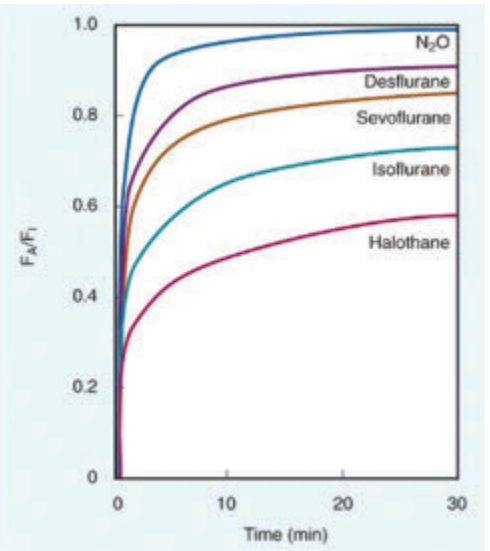

Figure 2. "Wash in" of different inhalational agents depending on their solubility in patients with same CO and minute ventilation. (Modified from Yasuda N, Lockhart SH, Eger EI 2nd, et al. Comparison of kinetics of sevoflurane and isoflurane in humans. *Anesth Analg.* 1991; 72:316–324) (14).

The rate of rise of F_A/F_I depends on the rate of delivery of anesthetic to the lungs and from the rate of uptake of anesthetic from the lungs to the bloodstream. There are six factors (listed in Table 2) that determine the wash-in of the inhaled anesthetics.

Table 2. Determinants of the Wash In.

Inspired concentration	Delivery of anesthetics TO the lungs
Alveolar ventilation	
Functional residual capacity	
Cardiac output	Delivery of anesthetics FROM the lungs to the blood
Solubility	
Alveolar-venous partial pressure gradient	

Increasing the **inspired concentration** of the anesthetic leaving the anesthesia machine by setting the vaporizer and the fresh gas flow, will increase its alveolar concentration and its rate of rise (F_A/F_I). This effect is also known as *concentration effect.*

The ratio of **alveolar ventilation** (Va) to **functional residual capacity** (FRC) is the main determinant for the delivery of anesthetics to the lungs, especially for the anesthetics that are more soluble. Va/FRC ratio is one of the differences between the rate of induction in adults and neonates. In neonates this ratio is 5:1, whereas in adults is 1.5:1 (higher ratio – faster rate of rise of FA/FI). The functional residual capacity is part of the breathing circuit and greater FRC means greater volume will be needed to be saturated with gas and more time for reaching equilibration between F_A and F_I (Figure 3).

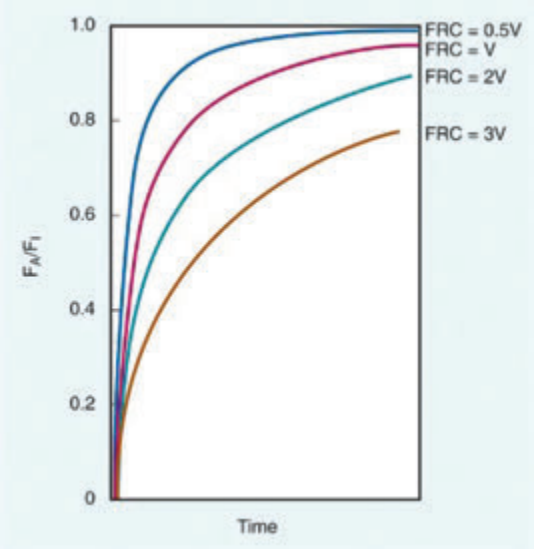

Figure 3. Correlation between different FRCs and the wash-in (FA/FI) in patients with the same cardiac output and minute ventilation (15).

For example, obese patients have lower FRC and the induction with inhalational anesthetics will be faster.

The changes in **cardiac output (CO)** are inversely related to the rate of rise of F_A/F_I (Figure 4). Patients with lower CO, like patients with heart failure, have lower blood flow through the lungs

and the extraction of the gas from the alveoli into the blood is lower, so the rate of decrease of F_A will be slower. This will lead to a faster rate of rise of F_A/F_I and a faster induction. In contrast, patients with high CO (anxiety), have faster uptake of anesthetic from the alveoli into the bloodstream, so the decrease of F_A is faster, and the rate of rise of F_A/F_I is slower.

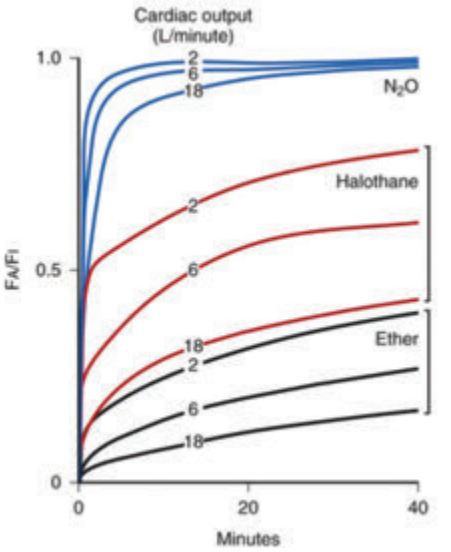

Figure 4. Correlation between CO and wash in rate in inhalational anesthetics with different solubility (16).

As mentioned before, partial pressure gradient of the gas between two phases is the driving force for anesthetics to move from the alveoli to the bloodstream and from the blood to the tissue. In the moment of equilibration of these partial pressures, the partial pressure of the anesthetic in the venous blood that returns to the heart will become equal to the partial pressure of the gas in alveoli. At that moment **alveolar – venous partial pressure gradient** is diminished and the uptake of the gas from the alveoli stops.

The solubility of anesthetics in blood and different tissues are expressed as partition coefficients, ratio of the anesthetic distributed between two phases when the partial pressures are equal. It actually defines the affinity of the anesthetic for one particular tissue (Table 3).

Table 3. Different partition coefficients at temperature of 37°C for different inhalational anesthetics.

	Blood: Gas	Brain: Blood	Fat: Blood
Nitrous oxide	0.47	1.1	2.3
Halothane	2.5	2.9	60
Methoxyflurane	12	2	49
Enflurane	1.9	1.5	36
Isoflurane	1.4	2.6	45
Desflurane	0.45	1.3	27
Sevoflurane	0.65	1.7	48

A blood: gas partition coefficient of 0.65 for sevoflurane means that when the partial pressures of sevoflurane are in equilibrium, the concentration of sevoflurane in the alveolus is 1 and 0.65

in blood. This means that sevoflurane has low solubility in blood which leads to fast induction and recovery from anesthesia. In this case, the blood reservoir is small, and the anesthetic can pass into/out of the brain quicker. While, for halothane blood: gas partition coefficient is 2.5 and means that when the partial pressures of halothane are in equilibrium, the concentration in the alveolus is 1 and 2.5 in blood. Blood acts as a reservoir (store) for the drug, so it does not enter or leave the brain until the blood reservoir is filled with drug. Halothane has high solubility in blood which leads to slow induction and recovery. As you can see from Figure 2 and Table 3, nitrous oxide has the fastest rate of rise od F_A/F_I although desflurane has lower solubility (0.45 vs 0.47 for N_2O). That is due to the concentration effect (we use 50-70% inspired concentration of N_2O compared to 6% desflurane).

High solubility does not mean high potency. The potency of the inhalational anesthetics is expressed through minimal alveolar concentration (MAC). MAC is defined as concentration of the anesthetics which prevent movement in 50% of the patients in response to surgical incision under standard conditions (atmospheric pressure and room temperature). MAC is analogous to ED_{50} in intravenous drugs and is inversely proportional to the potency (lower MAC = higher potency) (Table 4).

Table 4. Inhalational anesthetics and their MAC values.

Agent	MAC	Potency
Methoxyflurane	0.16%	The most potent
Halothane	0.74%	
Isoflurane	1.17%	
Enflurane	1.7%	
Sevoflurane	2.05%	
Desflurane	6.0%	
Nitrous oxide	104%	The least potent

MAC values are additive between different inhalational agents. That means if we want to achieve 1MAC of sevoflurane, we can use 0.5 MAC nitrous oxide and 0.5 MAC sevoflurane. But this is not the case in children. 60% Nitrous oxide combined with sevoflurane or desflurane in children, decreases the MAC of sevoflurane only 20% and 26% for desflurane (17, 18).

MAC 0.3-0.4 = MAC awake is defined as concentration of the inhaled anesthetic on which response to the verbal commands are lost in 50% of the patients (awakening from anesthesia in absence of other agents). On this level amnesia occurs. MAC 1.3 is level on which immobility is achieved in 95% of the patients and is analogous to ED_{95} in intravenous drugs. MAC is inversely related to lipid solubility, that is, if the lipid solubility decreases, the potency decreases and MAC increases.

The distribution or tissue uptake is managed by the same factors as the uptake of the anesthetic from the alveoli to the blood. These factors are tissue blood flow, tissue solubility (tissue: blood partition coefficient) and arterial blood-tissue partial pressure gradient. Tissues are classified in four groups according to their blood flow: vessel-rich group (VRG) (brain, heart, kidney, lung, liver), lean group (muscle and skin), vessel-poor group (connective tissue, bones) and fat (Table 5).

Table 5. Tissue classification according to their blood flow.

	Body mass (%)	CO(%)
Vessel-rich group	10	75
lean group	50	20
vessel-poor group	20	<1
fat	20	5

Since vessel-poor group receives small percentage of cardiac output, the distribution and equilibration of the inhalational anesthetic is happening in only three groups (VRG, muscle and fat). Equilibration happens when the anesthetic partial pressures in blood and tissues approaches that to the alveoli. The rate (time) at which this equilibration takes place is expressed as time constant (**τ**).

$$\tau_{tissue} = \frac{Volume\ of\ the\ tissue(ml)\ X\ tissue{:}blood\ solybility}{tissue\ blood\ flow\ (ml/min)}$$

1 **τ** is the time for 63-67% equilibration of partial pressures between tissue and the blood and for 98% equilibration is needed 3 – 4 times constants. Knowing that VRG receives 75% of cardiac output, time constant for this group (including the brain) is short and the equilibration is the fastest. Time constant for fat is very slow due to the high fat: blood partition coefficients of the different anesthetics (see Table 1). Only nitrous oxide has similar partition coefficients in all phases. More soluble agents have longer time constant. In order fat to be saturated with the gas, more time is needed to equilibration to be achieved (anesthesia should least more than 4 hours).

All inhalational anesthetics, with exception of nitrous oxide and xenon, are metabolized in liver in different degrees. They are metabolized via cytochrome P450 enzymes in the liver, mainly by CYP 2E1 (19). Halothane (up to 40% of absorbed dose), isoflurane (0.2%) and desflurane (0.02% of absorbed dose) are bio-transformed to trifluoroacetate (TFA). TFA acts as a hapten and binds covalently to hepatocyte proteins causing hepatic injury (20). Sevoflurane is metabolized 2 – 5% in the liver and its main metabolite is hexafluoroisopropanol which does not have same antigenic characteristics as TFA. The inhalational anesthetics that undergo little metabolism have become more popular while those that are metabolized in larger percentage (halothane, methoxyflurane – 75% of absorbed dose) have become a past. Most of the anesthetics are eliminated via exhalation (wash out) through the lungs.

During the emergency, the inspired concentration of inhalational anesthetic is set to zero and the wash-out of inhalational anesthetics follows an exponential decay. The time of the wash-out (and speed of emergence) of the inhalational anesthetics, depends on **the duration of anesthesia** and **solubility of the anesthetic** (14). Due to their blood solubility, the speed of emergency follows the order: desflurane > sevoflurane > isoflurane > halothane > methoxyflurane, especially if the fixed MAC is maintained till the end of the surgery (18). Also, the speed of emergency in parallel depends on the duration of anesthesia. Differences between various inhalational agents are less if the duration of anesthesia is short (Figure 5) (21).

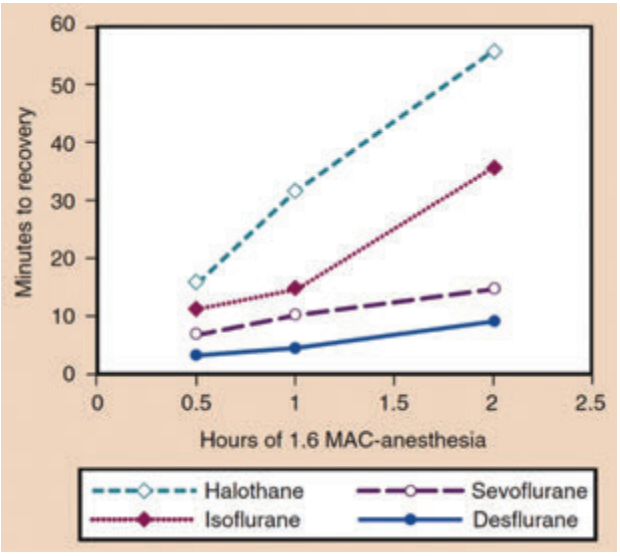

Figure 5. The time of recovery increases parallel to the duration of the anesthesia (22).

There are a few strategies that can be used to speed the recovery from anesthesia. Discontinuing nitrous oxide accelerates the wash-out of inhalational anesthesia due to the second gas effect that will be mentioned later (23). Charcoal filters have been shown that can speed the emergence by absorbing the anesthetics from the anesthesia breathing circuits (24). Hypercapnic hyperventilation together with charcoal filter has been shown to speed recovery from isoflurane, sevoflurane and desflurane anesthesia by near 60% (25). Related data for children are missing.

Special Factors

Two types of shunts occur: left-to-right, and right-to-left shunt. A left-to-right shunt happens in conditions where the blood from the heart recirculates into the lungs due to some intracardiac defect. This shunt has clinical significance for pharmacokinetics of the intravenous drugs and does not affect the PK of the inhalational drugs. A right-to-left shunt happens when the venous blood bypasses the lungs and returns to the heart. These conditions can be with intracardiac etiology (due to cyanotic heart disease) or intrapulmonary etiology (due to pneumonia or endobronchial intubation). A right-to-left shunt decreases the rate of wash in, especially for the less soluble anesthetics (sevoflurane, desflurane), so the induction is slower (26, 27). In these circumstances, when inhalational anesthetics are used for induction, intravenous anesthetics are required in order to achieve satisfactory depth of anesthesia.

There are a few factors that affect the faster wash-in (induction) in infants and children compared to adults. Infants have a greater ratio of alveolar ventilation to functional residual capacity (Va/FRC), which is 5:1 vs 1.5:1 in adults. A greater fraction of cardiac output is delivered to the vessel-rich group tissues in infants. Vessel – rich group constitutes 18% of the body weight in infants compared to 10% in adults. Infants have lower solubility in blood (lower tissue: blood and blood: gas partition coefficients) than in adults, due to the lower serum cholesterol and protein levels. But blood solubility of less soluble inhalational anesthetics such as sevoflurane, is similar in infants and adults (28).

Chemical Degradation

Sevoflurane undergoes chemical degradation in carbon dioxide absorbents to produce vinyl ether compound A. The production is greater in closed circuit breathing system, low flow and by warm and dry CO_2 absorbents (29). Barium hydroxide lime produces more compound A, compared to soda lime due to higher absorbent temperature during CO_2 extraction (30).

Compound A causes renal tubular necrosis in rats (31). Several studies have shown that sevoflurane in closed system with low flow produces compound A nearly 8 to 24ppm and 20 to 32ppm with soda lime and barium hydroxide lime, respectively (32, 33). Prospective, multicenter, randomized study conducted in 2002, in patients with previous renal disease, showed that there were no adverse renal effects of long duration, low-flow sevoflurane (34). Most of the countries that have approved sevoflurane for clinical use have no flow restriction, perhaps because of the proven safety of sevoflurane in scientific studies. Doses of compound A up to 400ppm per hour have no toxically renal effect, even in low flow anesthesia (0.5-1L/min) (35). This nephrotoxicity is dependent on species.

Interaction between volatile anesthetics and dry CO_2 absorbents can produce carbon monoxide. Factors that increase CO production include increased temperature and lower fresh gas flow, higher dryness of the absorbent and higher anesthetic concentration. Generally, temperatures in CO_2 canister are 25°C to 45°C, but can be higher when using a low fresh gas flow. Significant CO production with sevoflurane is noted if the canister temperature exceeds 80°C due to exothermic reaction (36). Desflurane and isoflurane conducted with CO_2 absorbents maintained at room temperature, 1 MAC desflurane produced up to 8,000ppm of CO versus 79ppm with nearly 2 MAC sevoflurane (37). Also, desflurane conducted with barium hydroxide has 3 folds higher CO production than conducted with soda lime. CO poisoning is difficult to be diagnosed because it is masked by anesthesia and pulse oximetry is unchanged (carboxyhemoglobin can't be detected by the pulse oximetry). Carboxyhemoglobin level can range up to 40%. The production of carbon monoxide is minor with sevoflurane and halothane, intermediate with isoflurane and maximum with desflurane and enflurane.

Modern CO_2 absorbents, based on lithium hydroxide, have been discovered in order to minimize production of compound A and carbon monoxide. Although, these absorbents are expensive, they can be used much longer than the previous absorbents.

N_2O: Concentration Effect, Second Gas Effect, Diffusion Hypoxia and Greenhouse Effect

As mentioned before, increasing the concentration of anesthetics in the inspired fraction (F_I) increases the concentration of anesthetics in the alveoli (F_A), and alveolar concentration faster approaches to the inspired concentration. This is known as **concentration effect** and has clinical relevance only for agents administrated at high concentrations (nitrous oxide and xenon). Nitrous oxide, administrated in high concentration, is quickly taken up into the bloodstream. The absorbed N_2O is substituted with proportional volume of gas which leads to faster rise of F_A (fractional concentration of anesthetic in alveoli) of nitrous oxide.

Combining N_2O with the second more potent gas on induction, can increase the wash-in of the second inhalational anesthetic. This effect is called the **second gas effect**. For example, during induction 1% of inhalational anesthetic is delivered in 80% N_2O and 19% oxygen. N_2O, due to high partial pressure and low solubility, is delivered into the blood more rapidly than the other inhalational anesthetic, and the alveolar N_2O concentration is decreased (e.g., by 50%). So, the uptake of N_2O is 40 parts (50% from 80% in the inspired fraction of N2O), leaving 40 parts N_2O, 19 parts O_2, and 1% second gas in the alveoli. The second gas now in the alveoli is present at a concentration of 1/ (1 + 40 + 19) = 1.7%, and oxygen is present 19/ (1+40=19) = 31.6% (Figure 6). The second gas (potent inhalational anesthetic) has been concentrated, F_A is increased and rate of rise of F_A/F_I is faster, which speeds the induction.

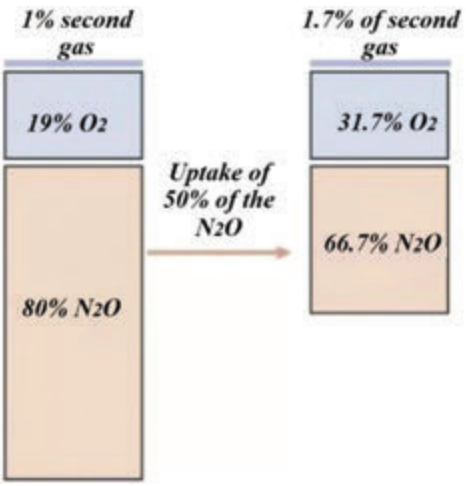

Figure 6. The second gas effect (38).

During the emergency, when N_2O is discontinued, it diffuses from blood into the alveoli very rapidly diluting alveolar oxygen. This can cause **diffusion hypoxia**. It can be avoided by increasing the inspired fraction of oxygen to 100% during the initial recovery.

Blood: gas partition coefficient of nitrous oxide is 0.47, whereas nitrogen blood: gas partition coefficient is 0.015, which means N_2O is 30 times more soluble in blood than nitrogen. Hence, nitrous oxide diffuses more rapidly in spaces with gas that contain nitrogen than nitrogen diffuses out of them. Closed air spaces like middle ear, pneumothorax, bowel, air emboli and tracheal tube cuff can be distended due to the diffusion of N_2O in them. This distention is time dependent but can be very dangerous in presence of air emboli, causing life-threatening air embolism.

All inhalational anesthetics contribute to global warming, except xenon which is an inert gas. Isoflurane, desflurane and sevoflurane are metabolized in very small percentage (0.2%, 0.02% and 2%, respectively) and after exhalation these agents remain in form that can pollute the environment. These are very powerful greenhouse gases, which can trap radiation and heat in the atmosphere until they undergo degradation in the atmosphere. The atmosphere lifetime varies between different inhalational agents: desflurane 14 years, isoflurane 3 years, sevoflurane 2 years and nitrous oxide can stay in the atmosphere for 114 years (39, 40).

Global warming potential (GWP) is measure of how much a greenhouse gas contributes to global warming over a period of 100 years, compared to equivalent mass of carbon dioxide

(GWP of CO_2 is one). The highest GWP_{100} has desflurane and is 2540, followed by isoflurane which value is 539, then nitrous oxide has GWP_{100} = 273 and sevoflurane is 144 times more potent than carbon dioxide as greenhouse gas (39). These factors are routinely updated as atmospheric chemistry is continuously changing. But we often overlook clinical potency when we think about GWP and the greenhouse emission. Desflurane has the lowest clinical potency (MAC 6.0%) of the volatile drugs, which means that to achieve an equivalent clinical effect at similar fresh gas flow rates as sevoflurane (MAC 2.0%) or isoflurane (MAC 1.2%), it requires three-to-five times higher concentration than sevoflurane or isoflurane, respectively. Although N_2O has a lower GWP_{100} than isoflurane (273 vs 539), it is usually delivered at a concentration of 50-70%, resulting in a higher overall environmental effect (40). According to the research done by Global Carbon Project, from 1980 to 2020, N_2O emission rose by 40% (41). Also, N_2O is the most anesthetic agent responsible for ozone depletion.

In order to protect our environment, scavenging systems must be used to prevent waste gas accumulation (WGA). The American Society of Anesthesiologists recommends several strategies in order to reduce these greenhouse gases emissions, including the following: consider total intravenous anesthesia and regional anesthesia or at least low flow anesthesia (use low fresh gas flow), avoid desflurane and nitrous oxide (high impact inhalational anesthetics), use portable tanks of nitrous oxide that remain closed between use instead centralized N_2O piping and investing in WGA trapping or WGA destroying technology (42).

References:

1. Long C.W. An account of the first use of sulphuric ether by inhalation as an anesthetic in surgical operations. South Med Surg J; 1849.
2. Marx T, Schmidt M, Schirmer U, Reinelt H. Xenon anesthesia. Journal of the Royal Society of Medicine. 2000; 93 (10): 513–517.
3. Eger EI 2nd. The pharmacology of inhaled anesthetics. J Crit Care. 2005; 24 (2):89–100.
4. Franks NP, Lieb WR. Where do general anesthetics act? Nature. 1978; 274 (5669):339–342.
5. Frazer MJ, Lynch C 3rd. Halothane and isoflurane effects on Ca^{2+} fluxes of isolated myocardial sarcoplasmic reticulum. Anesthesiology. 1992;77(2):316–323.
6. Narahashi T, Aistrup GL, Lindstrom JM, et al. Ion channel modulation as the basis for general anesthesia. Toxicol Lett. 1998;100-101:185–191.
7. Franks NP, Lieb WR. Molecular and cellular mechanisms of general anaesthesia. Nature. 1994;367(6464):607–614.
8. Zimmerman SA, Jones MV, Harrison NL. Potentiation of gamma-aminobutyric acid A receptor Cl- current correlates with in vivo anesthetic potency. J Pharmacol Exp Ther. 1994;270(3):987–991.
9. Yamakura T, Harris RA. Effects of gaseous anesthetics nitrous oxide and xenon on ligand-gated ion channels. Comparison with isoflurane and ethanol. Anesthesiology. 2000;93(4):1095–1101.
10. Nishikawa K, MacIver MB. Excitatory synaptic transmission mediated by NMDA receptors is more sensitive to isoflurane than are non-NMDA receptor-mediated responses. Anesthesiology. 2000;92(1):228–236.

11. Cordero-Erausquin M, Marubio LM, Klink R, Changeux JP. Nicotinic receptor function: new perspectives from knockout mice. Trends Pharmacol Sci. 2000;21(6):211–217.
12. Helge Eilers and Spencer Yost. General anesthetics. Basicmedical Key -https://basicmedicalkey.com/general-anesthetics.
13. Wilder RT, Flick RP, Sprung J, et al. Early exposure to anesthesia and learning disabilities in a population based cohort. Anesthesiology. 2009;110(4):796–804.
14. Yasuda N, Lockhart SH, Eger EI 2nd, et al. Comparison of kinetics of sevoflurane and isoflurane in humans. Anesth Analg. 1991; 72:316–324.
15. Pharmacokinetics of Inhaled Anesthetics. Anesthesia Key - https://aneskey.com/pharmacokinetics-of-inhaled-anesthetics.
16. Eger EI 2nd. Anesthetic Uptake and Action. Baltimore: Williams & Wilkins; 1974.
17. Taylor RH, Lerman J. Minimum alveolar concentration of desflurane and hemodynamic responses in neonates, infants, and children. Anesthesiology. 1991;75(6):975–979.
18. Lerman J, Sikich N, Kleinman S, Yentis S. The pharmacology of sevoflurane in infants and children. Anesthesiology. 1994; 80(4):814–824.
19. Kharasch ED, Thummel KE. Identification of cytochrome P450 2E1 as the predominant enzyme catalyzing human liver microsomal defluorination of sevoflurane, isoflurane, and methoxyflurane. Anesthesiology. 1993; 9:795–807.
20. Restrepo JG, Garcia-Martín E, Martínez C, et al. Polymorphic drug metabolism in anaesthesia. Curr Drug Metab. 2009; 10:236–246.
21. Sakata DJ, Gopalakrishnan NA, Orr JA, et al. Hypercapnic hyperventilation shortens emergence time from isoflurane anesthesia. Anesth Analg. 2007;104(3):587–591.
22. Eger EI II. Desflurane (Suprane): A Compendium and Reference. Nutley, NJ: Anaquest; 1993:1-11.
23. Peyton PJ, Chao I, Weinberg L, et al. Nitrous oxide diffusion and the second gas effect on emergence from anesthesia. Anesthesiology. 2011; 114(3):596–602.
24. Chang DJ, Choi SH, Choi YS, Min KT. Effect of charcoal filter on the emergence from sevoflurane anesthesia in a semi-closed rebreathing circuit. Yonsei Med J. 2011; 52(4):668–672.
25. Sakata DJ, Gopalakrishnan NA, Orr JA, et al. Hypercapnic hyperventilation shortens emergence time from isoflurane anesthesia. Anesth Analg. 2007;104(3):587–591.
26. Tanner GE, Angers DG, Barash PG, et al. Effect of left-to-right, mixed left-to-right, and right-to-left shunts on inhalational anesthetic induction in children: a computer model. Anesth Analg. 1985; 64(2):101–107.
27. Huntington JH, Malviya S, Voepel-Lewis T, et al. The effect of a right-to-left intracardiac shunt on the rate of rise of arterial and end-tidal halothane in children. Anesth Analg. 1999; 88(4):759–762.
28. Malviya S, Lerman J. The blood/gas solubilities of sevoflurane, isoflurane, halothane, and serum constituent concentrations in neonates and adults. Anesthesiology. 1990; 72(5):793–796.

29. Fang ZX, Kandel L, Laster MJ, et al. Factors affecting production of compound A from the interaction of sevoflurane with Baralyme and soda lime. Anesth Analg. 1996; 82:775–781.
30. Frink EJ Jr, Malan TP, Morgan SE, et al. Quantification of the degradation products of sevoflurane in two CO_2 absorbents during low-flow anesthesia in surgical patients. Anesthesiology. 1992; 77:1064–1069.
31. J. L. Martin, L. Kandel, M. J. Laster, R. L. Kerschmann, E. I. Eger II.Studies of mechanism of nephrotoxicity of Compound A in rats. Journal of Anesthesia. 1997; 11:32-37.
32. Bito H, Ikeda K. Closed-circuit anesthesia with sevoflurane in humans. Effects on renal and hepatic function and concentrations of breakdown products with soda lime in the circuit. Anesthesiology. 1994; 80:71–76.
33. Kharasch ED, Frink EJ Jr, Zager R, et al. Assessment of low-flow sevoflurane and isoflurane effects on renal function using sensitive markers of tubular toxicity. Anesthesiology. 1997; 86:1238–1253.
34. Litz RJ, Hübler M, Lorenz W, et al. Renal responses to desflurane and isoflurane in patients with renal insufficiency. Anesthesiology. 2002; 97:1133–1136.
35. Ebert TJ, Frink EJ Jr, Kharasch ED. Absence of biochemical evidence for renal and hepatic dysfunction after 8 hours of 1.25 minimum alveolar concentration sevoflurane anesthesia in volunteers. Anesthesiology. 1998; 88:601–610.
36. Holak EJ, Mei DA, Dunning MB 3rd, et al. Carbon monoxide production from sevoflurane breakdown: Modeling of exposures under clinical conditions. Anesth Analg. 2003; 96:757–764.
37. Fang ZX, Eger EI 2nd, Laster MJ, et al. Carbon monoxide production from degradation of desflurane, enflurane, isoflurane, halothane, and sevoflurane by soda lime and Baralyme. Anesth Analg. 1995; 80:1187–1193.
38. Pharmacology Mentor. Available from: https://pharmacologymentor.com/inhalation-anesthetics. Accessed May 1, 2025.
39. Sulbaek Andersen MP, Nielsen OJ, Sherman JD. Assessing the potential climate impact of anaesthetic gases. Lancet Planetary Health. 2023.
40. Ryan SM, Nielsen CJ. Global warming potential of inhaled anesthetics: application to clinical use. Anesth Analg. Jul 2010; 111(1):92-98.
41. NOAA research. 2024. Available from: https://research.noaa.gov/nitrous-oxide-emissions-grew-40-percent-from-1980-to-2020-accelerating-climate-change/. Accessed May 1, 2025.
42. ASA Inhaled Anesthesia Climate Challenge:https://www.asahq.org/about-asa/governance-and-committees/asa-committees/environmental-sustainability/inhaled-anesthetic-challenge. Date of last update: April 21, 2025.

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MIGRATING PULMONARY ANEURYSM IN BEHÇET VASCULITIS

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Abstract

Behçet's disease (BD), also called Behçet's syndrome, is a rare multisystemic vasculitis that causes blood vessel inflammation throughout the body. It may affect multiple organs and less frequently involves the central nervous system, potentially resulting in thrombotic events and aneurysm formation, the most notably pulmonary artery aneurysms (PAA).

We present a case of a 44-years-old female who sought medical attention due to prolonged hemoptysis combined with chest pain.

Ascribed to these symptoms, initial computer tomography (CT) (native and arterial phases) revealed an oval mass in the left hilum. Subsequent CT angiography demonstrated an aneurysm of the left interlobar artery compressing the segmental bronchus with associated thrombus formation. The patient refused surgical intervention. One year later, a follow-up CT angiography showed the resolution of the initial aneurysm but revealed a new aneurysm in the right main pulmonary artery.

This case highlights the rare migratory nature of pulmonary aneurysms in Behçet vasculitis, underscoring the need for early diagnosis, imaging follow-up and interdisciplinary management.

Key Words: *Behçet Vasculitis; CT angiography; case report; vascular anomalies.*

Introduction

Behçet vasculitis is a rare systemic vascular inflammatory disorder resulting in aneurysms and thrombosis (1). Pulmonary artery aneurysms are serious complications, often leading to life-threatening hemoptysis. Chronic endothelial dysfunction is central to the pathogenesis of Behçet disease (2). The prothrombotic factors and impaired fibrinolysis contribute to aneurysm development and thrombus formation. Advanced imaging, particularly contrast-enhanced computer tomography (CT) angiography, plays a pivotal role in diagnosis (3).

The clinical heterogeneity of Behçet disease mandates vigilance among clinicians and radiologists. Contrast-enhanced CT angiography with a 15-seconds delay is a key tool for precise anatomical delineation (4).

Endothelial damage triggers local thrombosis, potentially leading to aneurysm rupture or embolization. Patients with Behçet disease often exhibit hypercoagulability with elevated levels of procoagulant factors and impaired fibrinolysis. This prothrombotic environment contributes to vessel thrombosis and aneurysm formation (5).

This case report aims to contribute to the literature by detailing the clinical presentation, imaging findings and management approach of a patient with Behçet vasculitis and migrating pulmonary aneurysms.

The differential diagnosis includes Hughes-Stovin Syndrome (HSS), a rare autoimmune disorder characterized by pulmonary artery aneurysms, hemoptysis, chest pain and recurrent fever. HSS, like Behçet disease, results from vasculitis causing arterial occlusion, aneurysm formation and venous involvement. The histology of the aneurysms in both conditions can be similar (1).

By presenting a detailed analysis of a real-world case, showing that most of the such cases in the epidemiologic picture were seen in Mediterranean, East European and Asian regions, we aim to enhance awareness among healthcare professionals, fostering early recognition and facilitating timely intervention to mitigate potential complications associated with venous insufficiency.

This case report not only contributes to the expanding knowledge base of vascular anomalies but also serves as a reference for future cases of Behçet syndrome.

Case Presentation

A 44-years-old female presented with complaints of recurrent hemoptysis and persistent chest pain. She had no history of significant trauma, deep vein thrombosis, coagulopathies or other predisposing vascular conditions. Her past medical history was unremarkable, and she denied recent infections or autoimmune disorders. Initial imaging was performed with a non-contrast chest CT, and an oval-shaped mass was revealed in the left hilum, raising suspicion for a vascular anomaly. Contrast-enhanced CT angiography further delineated the lesion as an aneurysm in the left interlobar pulmonary artery, measuring several millimeters in diameter. The aneurysm was compressing the adjacent segmental bronchus and was associated with thrombus formation extending into the segmental and subsegmental bronchi supplying the left lower lung lobe. Follow-up imaging (1 year later) was done: the previously noted left interlobar artery aneurysm was no longer visualized, suggesting possible spontaneous resolution or thrombosis. However, a new aneurysm was identified in the right main pulmonary artery. This new aneurysm was associated with pulmonary micro-thrombosis, raising concerns for an underlying systemic or vascular pathology predisposing to aneurysm formation. The patient was referred to rheumatologist for laboratory evaluation and systemic therapy initiation. Due to disease progression, consultation with a vascular surgeon in Vienna, Austria was arranged. However, the patient refused surgical intervention. Long-term management includes ongoing imaging surveillance and symptomatic treatment to mitigate further complications.

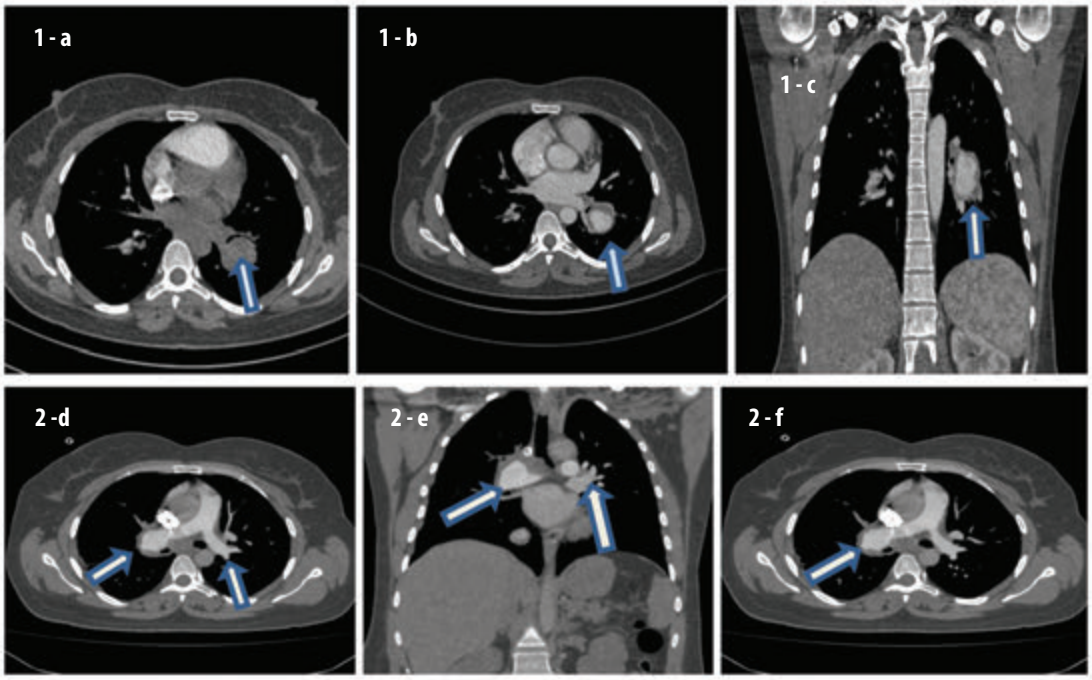

Figure 1a-c. The first three images depict aneurysm in the left interlobar artery Figure 1a-c: Aneurysm in the left interlobar artery (initial imaging).

Figure 2d-f. Migratory aneurysm in the right main pulmonary artery (follow-up imaging); the last 3 images are follow-up imaging.

Discussion

This report emphasizes the diagnostic value of CT angiography and the importance of long-term follow-up for patients with Behçet vasculitis. Pulmonary artery aneurysms in Behçet vasculitis are rare conditions, but they carry a high risk of rupture and fatal hemoptysis. The migratory nature of pulmonary aneurysms, as observed in this case, is an exceptionally rare phenomenon. Literature review suggests that Behçet vasculitis typically presents with multiple pulmonary artery aneurysms rather than the resolution of one with subsequent development of another at a different location. This case aligns with previous reports where aneurysmal thrombosis led to spontaneous resolution, while new vascular lesions emerged due to ongoing systemic inflammation (6,7).

A study by Yildirim and coauthors and another study by Giannessi, emphasized the role of CT angiography in detecting and monitoring vascular involvement in Behçet disease, particularly in patients with evolving aneurysms. The unpredictable nature of vascular involvement necessitates serial imaging for disease monitoring (6). Furthermore, a meta-analysis highlighted the efficacy of immunosuppressive therapy in reducing aneurysm progression, underscoring the need for early initiation of treatment (8).

Management strategies for Behçet-related pulmonary aneurysms include high-dose corticosteroids and immunosuppressive agents, such as cyclophosphamide or azathioprine, with or without anticoagulation. Endovascular intervention or surgical repair is reserved for cases with a high risk of rupture. In this case, the patient's refusal of surgical intervention underscores the need for individualized treatment plans, particularly in resource-limited settings.

Conclusion

This case highlights the migratory nature of pulmonary aneurysms in Behçet vasculitis, emphasizing the importance of early recognition, longitudinal imaging follow-up, and a multidisciplinary approach. Given the life-threatening potential of pulmonary aneurysms, timely diagnosis and intervention are crucial for optimizing patients' outcomes.

References:

1. Khan F, Gupta N, Khurana A, Zaheer S. Hughes-Stovin syndrome. Radiol Case Rep. 2017;12(3):550–3.
2. Bowness J, Nair R, Anwar S, et al. Pulmonary artery aneurysms in Behçet's syndrome. Clin Radiol. 2009;64(7):709–14.
3. Seyahi E, Melikoglu M, Akman C, et al. Pulmonary artery involvement in Behçet's disease. Clin Exp Rheumatol. 2008;26(4 Suppl 50): S27–33.
4. Seyahi E. Behçet's disease: How to diagnose and treat vascular involvement. Best Pract Res Clin Rheumatol. 2016;30(2):279–95.
5. Emmi G, Silvestri E, Squatrito D, et al. Behçet's syndrome as a model of thrombo-inflammation: From pathogenesis to treatment. Autoimmun Rev. 2022;21(11):103198.
6. Yıldırım R, Oğuzman S, Dinler M, Bilge NŞY, Kaşifoğlu T. Scoping beyond pulmonary artery involvement; pulmonary involvement in Behcet's disease; a retrospective analysis of 28 patients. Clin Rheumatol. 2023 Mar;42(3):849-853. doi: 10.1007/s10067-022-06423-5. Epub 2022 Nov 3. PMID: 36326947.
7. Giannessi C, Smorchkova O, Cozzi D, et al. Behçet's Disease: A Radiological Review of Vascular and Parenchymal Pulmonary Involvement. Diagnostics (Basel). 2022 Nov 19;12(11):2868. doi: 10.3390/diagnostics12112868.
8. Atalar E, Erten S, Dogan I, Konak HE. Vascular Involvement in Behcet's Disease: An Evaluation of 147 Cases and Literature Review. Sisli Etfal Hastan Tip Bul. 2023 Sep 29;57(3):380-386. doi: 10.14744/SEMB.2023.89083. PMID: 37900329; PMCID: PMC10600606.

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A BILIARY LEAK FROM DUCTS OF LUSCHKA AFTER LAPAROSCOPIC CHOLECYSTECTOMY - IMAGING FINDINGS

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Abstract

Acute cholecystitis is a condition which treatment usually involves surgery, and the most used is laparoscopic cholecystectomy. Bile leakage is a complication of laparoscopic cholecystectomy, often caused by injury to small aberrant bile ducts, such as the ducts of Luschka. We report a case of a 25-years-old female who was presented with abdominal pain five days after laparoscopic cholecystectomy. A CT scan and MRCP scan revealed a small bile collection in the gallbladder bed and the bile leakage was suspected. A laparoscopic revision was performed, during which a biliary collection was aspirated. Further exploration of the gallbladder fossa revealed an aberrant bile duct (duct of Luschka), smaller than 1mm, actively secreting bile. The duct was successfully closed, leading to resolution of the leakage. The ducts of Luschka are one of the most common causes of bile leakage after laparoscopic cholecystectomy. Generally, most of the diagnoses are determined post operatively as a result of the post-operative complications that arise. It is important to take into consideration the imaging reports whenever we have post cholecystectomy bile leakage.

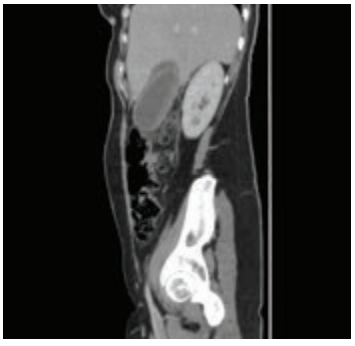
Key Words: *bile leakage; ducts of Luschka; laparoscopic cholecystectomy; magnetic resonance cholangiopancreatography*.

Introduction

Acute cholecystitis is an inflammation involving the gallbladder. Most commonly the reason is blockage of the cystic duct which leads to accumulation of bile, chemical injury, inflammation, hydrops and secondary bacterial infection of the gall bladder. About 95% of the patients with acute cholecystitis have cholelithiasis, only 5% don't have cholelithiasis. Usually the treatment involves surgery, the most commonly used is laparoscopic cholecystectomy which is a relatively safe procedure. The second most common complication that can arise is biliary tract leakage, which most often is associated with accessory ducts of Luschka or subvesical ducts that are an anatomic variation of the biliary ducts. Although they have important clinical impact, they can often be overlooked during routine imaging as the reason for the bile leakage. Radiological investigations that can be used are abdominal ultrasound (US), computed tomography (CT) scan and magnetic resonance cholangiopancreatography (MRCP) (1-5).

Case Report

A 25-years-old female patient was presented to the emergency department with nausea, vomiting and pain under the right rib cage for 4 days. On clinical examination, the abdomen was soft, but tenderness was noted in the right upper quadrant. Laboratory results showed elevated white cells, counting 17.5 (normal range: 4.00-9.00 10/9L), elevated CRP 82.8 (normal range: under 6mg/L), also elevated total bilirubin 23 (normal range under 20.5umol/L) and direct bilirubin 9.1 (normal range: under 6.8umol/L). Accordingly, an emergency CT scan of the abdomen with intravenous contrast was performed next, where the cholecyst was noted to have a stratified and edematous wall and intraluminal denser contents with a sediment formed in the fundus. Also a suspicious mural defect was present in the proximal part of the posterior wall. Pericholecystic free fluid was present that extended subhepatic with stranding of the mesenteric adipose tissue. Loco regionally several enlarged lymph nodes were detected with a diameter of up to 10mm. Free fluid was also present in the small pelvis. The CT finding was in favor of acute perforating cholecystitis.



FigurFe 1. Sagittal enhancing computed tomography iFFFmage of the abdomen revealing inflammatory changes of the gall bladder.

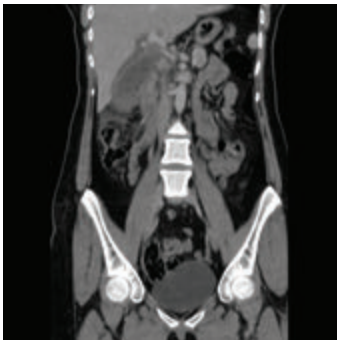


Figure 2. Coronal enhancing computed tomography image of the same level showing the same changes of the gall.

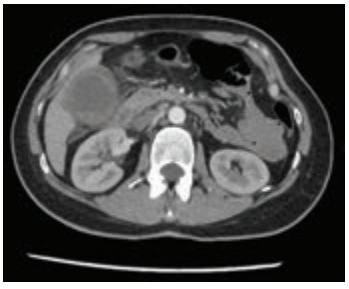


Figure 3. Axial enhancing computed tomography image at the level of the suspected perforation of the gall bladder.

A decision for surgical treatment was made. The procedure involved laparoscopic retrograde cholecystectomy with removal of the gallbladder through a supraumbilical incision, peritoneal lavage was performed, and intraperitoneal drain was placed. The patient was discharged in good general condition.

Five days after the operative treatment, the patient experienced severe abdominal pain accompanied by nausea and vomiting. The laboratory results showed elevated white cells count 11.1 (normal range: 4.00-9.00 10/9L), elevated CRP 67.3 (normal range: under 6mg/L), normal total bilirubin 14 (normal range under 20.5umol/L) and elevated direct bilirubin 10.2 (normal range: under 6.8umol/L). Control CT scan of the abdomen with intravenous contrast was performed, where in the region of the gall bladder a dense free liquid collection was observed, around which small free air inclusions were present, the clips of the cystic artery and ductus cysticus were also visible, and a smaller amount of free liquid was present in the pelvis.

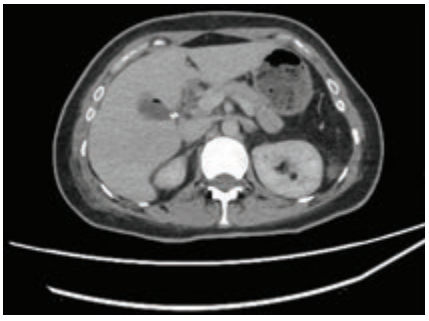


Figure 4. Axial enhancing computed tomography image revealing dense fluid collection in the gall bladder fossa with clips after surgery

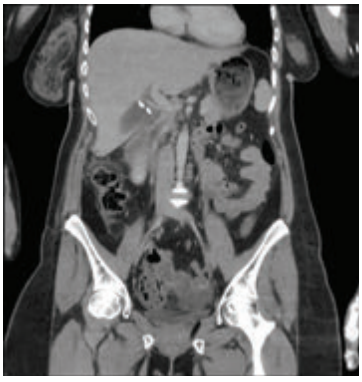


Figure 5. Coronal contrast enhancing computed tomography image of the same level with the same changes.

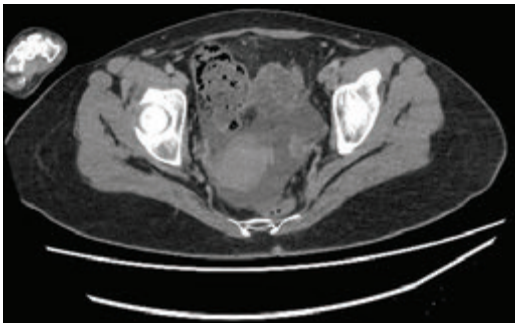


Figure 6. Axial contrast enhancing computed tomography image that shows free fluid was present in the pelvis.

An MRCP was performed next, that showed intact common bile duct and intact cystic duct stump. Also, an area of fluid accumulation was noted in the subhepatic space, intraintestinal and in the small pelvis. Subcutaneous oedema in the right lateral abdominal wall was also noted, most likely post operatively.

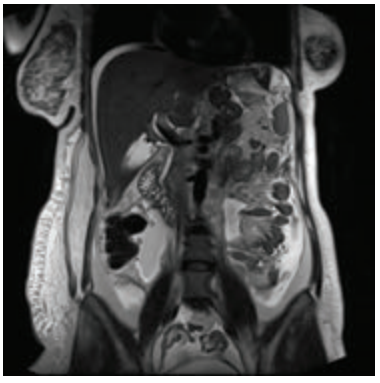


Figure 7. MRCP. T2 coronal image revealed intact CBD and intact cystic duct stump.

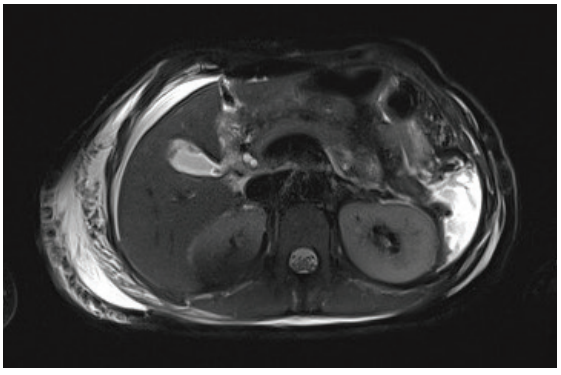


Figure 8. T2 FS axial image confirmed the CT finding of fluid collection in the gall bladder bed.

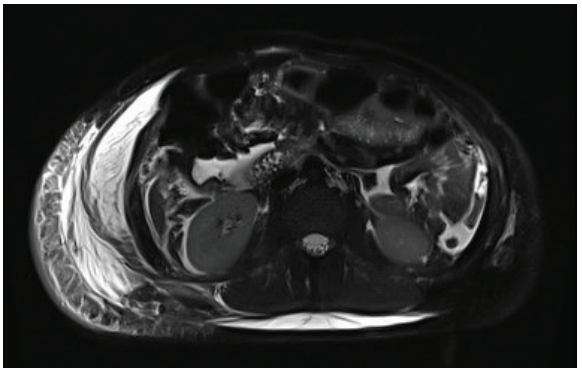


Figure 9. T2 FS axial image showing fluid accumulation in the subhepatic space, intraintestinal and subcutaneous oedema in the right lateral abdominal wall

An indication for postoperative laparoscopic revision was made where a larger biliary collection was found in the abdomen that was aspirated, and after a thorough exploration of the gall bladder bed, an aberrant bile duct smaller than 1mm was observed that is a Luschka subvesical duct, which secreted bile with an extremely small content. A biliary stasis was performed with a

ligation of the aberrant Luschka duct using sutures and two intraperitoneal drains were placed for drainage. The postoperative course was in order. The control CT of the abdomen and pelvis after 2 weeks showed complete resorption of the free fluid in the abdomen and pelvis. In the region of the gall bladder fossa, several more air inclusions were observed, which would be in favor of a normal postoperative reaction. The patient was discharged for home treatment in good general condition with advice given for a hygiene-dietary regimen and antibiotic therapy according to the protocol for operated patients.

Discussion

The accessory ducts of Luschka, also called subvesical bile ducts are small ducts usually with a diameter of around 1 to 2mm, that drain sub segmental areas of the liver into the right hepatic duct (6,7). The overall prevalence in the general population is around 4% (6). Due to their small caliber and especially in a urgent setting of laparoscopic cholecystectomy, they can be overlooked, which would lead to post-operative complications like bile leakage (8,9). Generally, most of the diagnoses are determined post operatively as a result of the post-operative complications that arise (8). Imaging methods that can be used are US (ultrasound), CT (computed tomography), MRCP (magnetic resonance cholangiopancreatography) and ERCP (endoscopic retrograde cholangiopancreatography). However, they present a diagnostic difficulty and most often they are overlooked (6,8). US is usually the initial diagnostic method, with a sensitivity of around 70% for detection of intra-abdominal bile collections, however it isn't very specific and is operator dependent (3,11-13). CT is more advanced method with a reported sensitivity of around 95% for detection of bile collection but cannot differentiate it from other fluid collections (6,10-13). ERCP is the most commonly used and is considered as the gold standard for the diagnosis of bile leakage after cholecystectomy. Its sensitivity is 95-100%. Also, it has high specificity of around 95%. With ERCP the sites of bile leakage can be evaluated with direct visualization of the contrast extravasation. However, this diagnostic procedure is invasive (9,12-14). MRCP is a non-invasive diagnostic procedure that can be used for assessing the anatomic structures of the biliary system with excellent detail. There are reported cases in literature, although uncommon when MRCP usually in combination with CT was successfully used for the diagnosis of bile leakage from Luschka ducts. Its sensitivity ranges from 67% to 100% and depends on many factors. Multidisciplinary approach is crucial for the fast detection of this complication after laparoscopic cholecystectomy and the radiologist should be aware of the diagnostic possibility of MRCP (6,10,11,15,16).

Conclusion

Imaging methods, especially CT and MRCP, play an important role in detecting and managing bile leaks after cholecystectomy. They help in identifying fluid collections and assess biliary anatomy and anatomic variations as the subvesical ducts of Luschka. It is important that the imaging reports take them into consideration whenever we have post cholecystectomy bile leakage, which will help in expediting the treatment of the patients.

References:

1. Jones MW, Genova R, O'Rouke MC. Acute Cholecystitis. In: StatPearls. Treasure Island (FL): StatPearls Publishing; May 22, 2023
2. Vitiello, A., Spagnuolo, M., Persico, et al. Biliary Leak from Ducts of Luschka: Systematic Review of the Literature. *Surgeries* 2024, *5*, 63-72. doi:10.3390/surgeries5010008.
3. Garcia S., Concepción AM., Wakoff C. "Bile Leak Due to Luschka Duct Injury After Laparoscopic Cholecystectomy: A Case Report." Cureus vol. 14,8 e28427. 26 Aug. 2022, doi:10.7759/cureus.28427.
4. Muleta J, Belayneh E, Haile K, et al. "A rare case of bile leak due to type 2 duct of Luschka injury post open cholecystectomy: a case report." Journal of surgical case reports vol. 2024,3 rjae179. 21 Mar. 2024, doi:10.1093/jscr/rjae179.
5. Bozer, A. "Hepatobiliary-Specific MRI Contrast Agent Detection of Subvesical Duct (Luschka's) Injury Post-Laparoscopic Cholecystectomy: A Case Report". Journal of the Belgian Society of Radiology, vol. 108, no. 1, 2024, p. 21, doi:10.5334/jbsr.3532.
6. Lin, Y. H., Lin, C. W., Yin, W. Y. "Subvesical Duct Detected by Magnetic Resonance Cholangiopancreatography (MRCP) in a Patient with Bile Leak after Laparoscopic Cholecystectomy." CRSLS: MIS case reports from SLS vol. 8,1 e2020.00074. 1 Apr. 2021, doi:10.4293/CRSLS.2020.00074.
7. Parampalli U., Helme S., Asal G., et al. Accessory cystic duct identification in laparoscopic cholecystectomy, Grand Rounds Vol 8 pages 40–42, DOI: 10.1102/1470-5206.2008.0014.
8. Selleslag, S., Vandeputte, M., Walgraeve, M.-S. "Unveiling the Hidden Culprit: A Case of Bile Leakage Post-Cholecystectomy Caused by a Luschka Duct". Journal of the Belgian Society of Radiology, vol. 107, no. 1, 2023, p. 59, doi:10.5334/jbsr.3233.
9. Gómez NA, Gutiérrez JO. Ducts of Luschka: A Review. Clin Surg. 2017; 2:1744.
10. Carannante F., Mazzotta E., Miacci V., et al. Identification and management of subvesical bile duct leakage after laparoscopic cholecystectomy: A systematic review, Asian Journal of Surgery, Volume 46, Issue 10, 2023, Pages 4161-4168, doi:10.1016/j.asjsur.2023.04.031.
11. Thomas S, Jahangir K. Noninvasive Imaging of the Biliary System Relevant to Percutaneous Interventions. Semin Intervent Radiol. 2016 Dec;33 (4):277-282. doi: 10.1055/s-0036-1592328.
12. Walker AT, Shapiro AW, Brooks DC, et al. Bile duct disruption and biloma after laparoscopic cholecystectomy: imaging evaluation. AJR Am J Roentgenol. 1992;158(4):785–9. doi: 10.2214/ajr.158.4.1532111
13. Yun SU, Cheon YK, Shim CS,et al. The outcome of endoscopic management of bile leakage after hepatobiliary surgery. Korean J Intern Med. 2017 Jan;32(1):79-84. doi: 10.3904/kjim.2015.165.
14. Kim K. H., Kim T. N. Endoscopic management of bile leakage after cholecystectomy: a single-center experience for 12 years. The Korean journal of internal medicine vol. 32,1 (2017): 79-84. doi:10.3904/kjim.2015.165.

15. Griffin N, Charles-Edwards G, Grant LA. Magnetic resonance cholangiopancreatography: the ABC of MRCP.Insights Imaging. 2012 Feb;3(1):11-21. doi: 10.1007/s13244-011-0129-9.

16. Argirò, R.; Sensi, B.;Siragusa, L.; et al. Liver-Specific Contrast-Enhanced Magnetic Resonance Cholangio-Pancreatography (Ce-MRCP) in Non-Invasive Diagnosis of Iatrogenic Biliary Leakage. Diagnostics 2023, 13, 1681. Doi :10.3390/diagnostics1310168

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MALIGNANT PERIPHERAL NERVE SHEATH TUMOR IN THE RETROPERITONEUM NOT ASSOCIATED WITH THE RENAL OR PERIRENAL PARENCHYMA

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Abstract

The occurrence of an isolated malignant peripheral nerve sheath tumor (MPNST) in the retroperitoneum, unassociated with the kidney capsule, is exceedingly rare, often presenting with an insidious onset of non-specific and misleading symptoms, primarily characterized by lower back pain.

We present the case of a patient with a malignant peripheral nerve sheath tumor (MPNST), without neurofibromatosis with nonspecific localization in the retroperitoneum in front of vena cava inferior non associated with the kidney. A 62-years-old male patient was referred due to the presence of an abdominal tumor without accompanying pain. Computed tomography identified a substantial soft tissue retroperitoneal tumor situated anterior to the inferior vena cava. A total resection was performed. The pathological testing verified the existence of a malignant peripheral nerve sheath tumor. The patient received additional treatment through radiotherapy and chemotherapy. This is the inaugural reported case of malignant peripheral nerve sheath tumor in the retroperitoneum without neurofibromatosis, unassociated with renal or perirenal parenchyma. We recommend incorporating malignant peripheral nerve sheath tumor into the differential diagnosis of abdominal masses.

Key Words: *Malignant peripheral nerve sheath tumor; prognosis; retroperitoneum, surgery.*

Introduction

Malignant peripheral nerve sheath tumors (MPNSTs) are rare soft-tissue sarcomas that commonly occur in the extremities and are often associated with neurofibromatosis. MPNSTs accounts for 5–10% of all soft-tissue sarcomas, and up to 50% occur in patients with neurofibromatosis type 1 (NF-1), with an incidence of 1 to 10 in 1000000 patients (1). MPNSTs affect adults aged 20–50 years and are typically connected to the main trunks of nerves. Most MPNSTs are aggressive and have high rates of recurrence and distant metastases, with the lungs being the

most common site of MPNST metastases (1,2). An MPNST of the kidney is an exceedingly rare occurrence. Only a few cases of MPNST in the retroperitoneum that is not associated with the kidneys have been reported in the literature (3). An aggressive surgical approach and combined chemotherapy are the accepted model of treatment (4).

Case Presentation

A 62-years-old male, a former smoker with a known history of hypertension managed with Enalapril 5mg once daily, obesity (body mass index: 34.6kg/m^2), and benign prostatic hyperplasia treated with Tamsulosin 0.4mg once daily, was incidentally found to have a retroperitoneal mass on abdominal ultrasound and computed tomography (CT). The patient was asymptomatic at the time of presentation and his physical examination was unremarkable. There was no personal or familial history of von Recklinghausen's disease.

Laboratory investigations revealed hemoglobin (Hb) of 108g/L, leukocytes (Le) at 6.3 x 10^9/L, erythrocytes (Er) at 5.01 x 10^{12}/L, hematocrit (Hct) at 0.352L/L, platelets (Tr) at 320 x 10^9/L, fasting glucose at 5.17mmol/L, urea at 7.6mmol/L, creatinine at 77.6µmol/L, sodium (Na) at 140mmol/L, potassium (K) at 3.6mmol/L, chloride (Cl) at 106mmol/L, and calcium (Ca) at 2.28mmol/L. A chest radiograph demonstrated findings consistent with chronic bronchitis. Abdominal CT confirmed the presence of a 9x9cm retroperitoneal soft tissue mass, predominantly solid, with distal sections containing a small amount of fluid. The lesion was positioned anteriorly to the inferior vena cava, extending towards the right hepatic lobe and the quadrate lobe of the liver, without definitive imaging evidence of hepatic origin. Given the deep location of the lesion, percutaneous biopsy under CT guidance was deemed unsuitable. The patient was scheduled for tumor resection and presented to the operating room in an awake and alert state, albeit with mild preoperative anxiety. Pre-induction vital signs were as follows: blood pressure (BP) 182/98mmHg, heart rate (HR) 95bpm, respiratory rate (RR) 20 breaths per minute, and oxygen saturation (SaO2) 97%. Standard monitoring, including electrocardiographic (ECG) leads, non-invasive blood pressure measurement, and peripheral oximetry, was applied. A thoracic epidural catheter was placed at the Th12-L1 interspace and tested with 2mL of 0.5% bupivacaine, which yielded a negative response. Preoxygenation was performed with 100% oxygen via a face mask, and the patient was premedicated with intravenous midazolam (2mg). Anesthesia induction was achieved with fentanyl (0.2mg), propofol (200mg), and rocuronium (50mg). Cricoid pressure was applied, and an 8.5mm cuffed endotracheal tube was successfully inserted. Post-induction hemodynamics remained elevated, with BP ranging from 160-180/95-100mmHg and HR at 90-95bpm, with a normal sinus rhythm. Anesthesia was maintained with a mixture of air and oxygen, supplemented with sevoflurane at 0.6-0.7 minimum alveolar concentration (MAC). Analgesia was provided using fentanyl (0.3mg) in divided doses, along with epidural administration of fentanyl (0.1mg) and 8mL of 0.25% bupivacaine. However, 20-30 minutes into the procedure, a progressive rise in both systolic and diastolic blood pressure was observed, accompanied by sinus tachycardia (HR 110-120bpm). Blood pressure escalated to 270/130mmHg, with intermittent premature ventricular contractions detected on ECG. Initial management included intravenous enalapril (1.25mg), which was ineffective. Consequently, an infusion of remifentanil (2mg/40mL) was initiated at 0.125mg/kg/min, alongside intravenous administration of esmolol (10mg), magnesium sulfate (15%, 0.3mg), and glyceryl trinitrate (0.5mcg/kg/min). Upon tumor removal, the patient experienced a precipitous drop in systolic BP to 50mmHg. Immediate resuscitative measures included administration of prednisolone (100mg), ephedrine (9mg), and 500mL of 6% hy-

droxyethyl starch (130/0.4), which restored BP to 110/60mmHg. The procedure was completed without major intraoperative blood loss. The patient was subsequently administered atropine (1mg) and neostigmine (2.5mg). Following adequate spontaneous respiration and response to stimuli, extubating was performed, and the patient was transferred to the postoperative recovery unit. During the immediate postoperative period, the patient's BP remained within normal limits. After two hours of monitoring, he was discharged to the Department of Urology. The patient recovered uneventfully and was discharged home on the fifth postoperative day. He was subsequently referred to the Clinic of Oncology for adjuvant radio-chemotherapy. Surgical Findings and Histopathological Examination reviled resected tumor mass weighed 150g and measured 9.5×7.5×5cm. The encapsulated lesion exhibited a soft consistency. Gross pathology revealed a gray-yellowish tumor with a well-demarcated, cystic lumen (3cm in diameter) containing blood. Microscopic examination identified a malignant mesenchymal neoplasm composed of epithelioid cells with abundant eosinophilic cytoplasm and pleomorphic to bizarre nuclei. Tumor necrosis was observed. Immunohistochemical analysis demonstrated positivity for vimentin, while markers for desmin, CD117 and CD34 were negative. Based on these findings, the final diagnosis was malignant peripheral nerve sheath tumor (MPNST), epithelioid variant.

Discussion

Malignant peripheral nerve sheath tumors (MPNSTs) are exceptionally rare, with an incidence ranging from 1 to 10 per 1,000,000, accounting for approximately 3% to 12% of all soft tissue sarcomas. These tumors predominantly arise in patients between 20 and 50 years of age, with common locations including the head and neck, trunk, or extremities (1,2,5). While MPNSTs in the retroperitoneal area are uncommon, several case reports have documented their occurrence (3,6). The prognosis for MPNSTs is generally poor, with reported five-years disease-specific survival rates ranging from 16% to 60% (6). Surgical resection followed by adjuvant radiotherapy remains the most effective treatment modality (5,7). Contrast-enhanced computed tomography (CT) and magnetic resonance imaging (MRI) play a critical role in the assessment of MPNSTs, allowing for precise delineation of tumor extent and suggesting a neurogenic origin (1). In our presented case, MRI findings indicative of malignancy include heterogeneity, areas of necrosis and hemorrhage, and increased uptake on positron emission tomography (PET). Given the aggressive nature of these tumors, a multidisciplinary approach is essential to ensure complete surgical excision and optimal patient's outcomes (1,7). Histological and immunohistochemical analysis are crucial for an accurate diagnosis of MPNST. Grossly, these tumors appear fusiform, typically exceed 5 cm in diameter, and exhibit a gray-tan coloration (8). Those findings were revealed in our presented case. Microscopically, MPNSTs display spindle cell morphology with a fascicular pattern, varying degrees of mitosis, necrosis and occasional tumor calcification (9). Immunohistochemical markers, including S-100 protein, neuron-specific enolase, actin, cytokeratin (CK), smooth muscle actin (SMA), desmin and vimentin, help differentiate MPNST from other spindle cell sarcomas. MPNSTs are often highly aggressive, demonstrating a high propensity for local recurrence and metastasis, even with multimodal therapy. Although these tumors frequently arise in patients with neurofibromatosis type 1 (NF-1), isolated cases without NF-1 have been reported. MPNSTs originating in the retroperitoneum, particularly those not associated with the kidney or perirenal structures, are exceedingly rare (10). Our case represents a retroperitoneal MPNST without renal or perirenal involvement and in the absence of NF-1. Similar cases in the literature underscore the aggressive nature of these tumors and their variable response to treatment. Costa et al. described an MPNST with diaphragmatic and pulmonary

invasion; despite surgical resection, the patient developed local recurrence and succumbed to the disease within 15 months (11). Jankulovski et al. reported an MPNST infiltrating the psoas muscle and left renal capsule, requiring radical nephrectomy, with adjuvant radiotherapy and chemotherapy resulting in six-months disease-free survival (3). Longer survival has been noted in select cases. A case of renal MPNST with metastatic spread to the scalp, lung, and shoulder was managed with neoadjuvant doxorubicin and extensive surgical resection, achieving disease-free survival at two years (12). Similarly, patients with MPNST of the renal pelvis have demonstrated survival durations of 24 to 36 months following aggressive surgical intervention (13).

Despite advances in multimodal therapy, including radical surgical resection and adjuvant radiotherapy, the prognosis for MPNST remains poor, emphasizing the need for early diagnosis and comprehensive treatment planning to improve patient's outcomes (14).

Conclusion

Malignant peripheral nerve sheath tumors (MPNSTs) of the retroperitoneum, particularly those without renal or perirenal involvement, are exceedingly rare and present a significant diagnostic and therapeutic challenge. Our case is an accidental find of MPNST differential diagnostically suspected for suprarenal tumor and highlights the importance of early detection and a multidisciplinary approach in managing these aggressive tumors. Surgical resection remains the cornerstone of treatment, with adjuvant radiotherapy and chemotherapy playing a critical role in disease control. Despite optimal management, MPNSTs have a high propensity for recurrence and metastasis, underscoring the need for vigilant postoperative surveillance. Given the aggressive nature and poor prognosis associated with these tumors, further research into novel therapeutic strategies is essential to improve patient outcomes.

References:

1. John L, William M, Pictorial E. Cross-sectional imaging of peripheral nerve sheath tumors. Characteristic signs on CT, MR imaging, and sonography. AJR Am J Roentgenal 2001; 176:75–82.
2. Fuchs B, Spinner RJ., Rock MG.Malignant peripheral nerve sheath tumors: an update. J Surg Orthop Adv 2005; 14:168–74.
3. Jankulovski N, Stankov O, Banev S, et al. Isolated malignant peripheral nerve sheath tumor of kidney capsule. Contribution 2008; 2:361–369.
4. Cameron RB, Loehrer PJ, Thomas J. Neoplasms of the mediastinum. Cancer Principles and practice of Oncology 6th ed,. Lippincott Williams and Wilkins, USA (2001) 1031–1032.
5. Somatilaka BN, Sadek A, McKay RM, Le LQ. Malignant peripheral nerve sheath tumor: models, biology, and translation. Oncogene. 2022 Apr;41(17):2405-2421. doi: 10.1038/s41388-022-02290-1. Epub 2022 Apr 7. PMID: 35393544; PMCID: PMC9035132.
6. Seres R, Hameed H, McCabe MG, Russell D, Lee ATJ. The Multimodality Management of Malignant Peripheral Nerve Sheath Tumours. Cancers. 2024; 16(19):3266. https://doi.org/10.3390/cancers16193266.

7. Cai Z, Tang X, Liang H, Yang R, Yan T, Guo W. Prognosis and risk factors for malignant peripheral nerve sheath tumor: a systematic review and meta-analysis. World J Surg Oncol. 2020 Sep 30;18(1):257. doi: 10.1186/s12957-020-02036-x. PMID: 32998743; PMCID: PMC7528472.

8. Magro G, Broggi G, Angelico G, Puzzo L, Vecchio GM, Virzì V, Salvatorelli L, Ruggieri M. Practical Approach to Histological Diagnosis of Peripheral Nerve Sheath Tumors: An Update. Diagnostics (Basel). 2022 Jun 14;12(6):1463. doi: 10.3390/diagnostics12061463. PMID: 35741273; PMCID: PMC9222088.

9. Kar M, Deo SV, Shukla NK, Malik A, DattaGupta S, Mohanti BK, Thulkar S. Malignant peripheral nerve sheath tumors (MPNST)--clinicopathological study and treatment outcome of twenty-four cases. World J Surg Oncol. 2006 Aug 22;4:55. doi: 10.1186/1477-7819-4-55. PMID: 16923196; PMCID: PMC1560134.

10. Miettinen M. Immunohistochemistry of soft tissue tumours - review with emphasis on 10 markers. Histopathology. 2014 Jan;64(1):101-18. doi: 10.1111/his.12298. Epub 2013 Nov 28. PMID: 24111893; PMCID: PMC7670586.

11. Costa R, Maximo J, Fernandes P, Guimarães S, Silva R, Magalhães A. A rare case of pulmonary malignant peripheral nerve sheath tumor. Pulmonology. 2023 Nov-Dec;29(6):574-575. doi: 10.1016/j.pulmoe.2023.01.006. Epub 2023 Mar 10. PMID: 36907816.

12. Shurell-Linehan E, DiPardo BJ, Elliott IA, Graham DS, Eckardt MA, Dry SM, Nelson SD, Singh AS, Kalbasi A, Federman N, Bernthal NM, Eilber FC. Pathologic Response to Neoadjuvant Therapy is Associated With Improved Long-term Survival in High-risk Primary Localized Malignant Peripheral Nerve Sheath Tumors. Am J Clin Oncol. 2019 May;42(5):426-431. doi: 10.1097/COC.0000000000000536. PMID: 30913092.

13. Zhang L, Lemberg KM, Calizo A, Varadhan R, Siegel AH, Meyer CF, Blakeley JO, Pratilas CA. Analysis of treatment sequence and outcomes in patients with relapsed malignant peripheral nerve sheath tumors. Neurooncol Adv. 2023 Dec 2;5(1):vdad156. doi: 10.1093/noajnl/vdad156. PMID: 38130899; PMCID: PMC10733661.

14. Somaiah N, Paudyal B, Winkler RE, Van Tine BA, Hirbe AC. Malignant Peripheral Nerve Sheath Tumor, a Heterogeneous, Aggressive Cancer with Diverse Biomarkers and No Targeted Standard of Care: Review of the Literature and Ongoing Investigational Agents. Target Oncol. 2024 Sep;19(5):665-678. doi: 10.1007/s11523-024-01078-5. Epub 2024 Jul 2. PMID: 38954182; PMCID: PMC11392982.

CASE REPORT *UDK: UDK: 616-056.7-06-008.857-036.81*

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A CRITICAL CARE PERSPECTIVE ON FAMILIAL MEDITERRANEAN FEVER

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Abstract

Familial Mediterranean Fever (FMF) is a rare autosomal recessive autoinflammatory disorder predominantly affecting individuals of Mediterranean origin. FMF is characterized by recurrent febrile episodes and serositis with amyloidosis being its most severe complication, often leading to end-stage renal disease (ESRD). The progression of FMF to amyloidosis is associated with increased morbidity and mortality requiring early diagnosis and effective management. This case report presents a critically ill patient with FMF, amyloidosis and vasculitis-related complications including massive hemorrhage and multi-organ failure. It highlights these complex conditions, the challenges in ICU management and the necessity of a multidisciplinary approach to optimize patients' outcomes.

Keywords: *amyloidosis; intensive care; Mediterranean fever; rare disease.*

Introduction

Familial Mediterranean Fever (FMF) is an inherited autoinflammatory disorder caused by mutations in the MEFV gene, located on chromosome 16 (16p13). This gene encodes a protein called pyrin, which plays a key role in the regulation of inflammation. Mutations in pyrin result in uncontrolled activation of the inflammatory response which leads to episodic fever, serositis, arthritis and long-term complications such as amyloidosis. Although FMF is rare globally, it's more prevalent in Mediterranean populations particularly among Turkish, Armenian, Arab and Sephardic Jewish communities with an incidence of approximately 1 in 1,000 individuals (1).

The most severe long-term complication of FMF is systemic amyloidosis which results from the deposition of amyloid A protein in various organs, particularly the kidneys. This deposition leads to progressive renal dysfunction and end stage renal disease (ESRD). The life expectancy of patients with FMF-associated amyloidosis is significantly reduced with survival ranging from 24 to 53 months after diagnosis (2). Furthermore, FMF has been linked to vasculitis disorders such as Henoch-Schonlein Purpura (HSP) and Polyarteritis Nodosa (PAN), both of which contribute to vascular inflammation and increased risk of hemorrhagic events. Amyloid deposition in blood vessel walls weakens the structural integrity of the tunica media and adventitia, leading to luminal stenosis, microaneurysms and a predisposition to vascular rupture and hemorrhage.

Case Presentation

A 49-years-old male with a known history of FMF and ESRD secondary to amyloidosis was admitted to the ICU with hemodynamic instability and altered mental status. He had been receiving regular hemodialysis (3-4 times per week) for renal failure. On admission, his vital signs were as follows: blood pressure 70/40mmHg, heart rate 110bpm and SpO2 86%. The patient was intubated and placed on mechanical ventilation due to respiratory distress and progressive deterioration in mental status.

Management

Day 1-3: The patient presented with septic shock, requiring aggressive fluid resuscitation and vasopressor support. Arterial blood gas analysis revealed severe metabolic acidosis (pH 7.1, HCO3- 12mmol/L, lactate 6mmol/L). Laboratory investigations showed severe anemia (Hb 6.2g/dL), thrombocytopenia (platelets 45,000/μL) and elevated inflammatory markers (CRP 145mg/L). The most concerning feature was massive epistaxis and oropharyngeal hemorrhage, which persisted despite normal coagulation parameters (PT, aPTT and INR within normal limits).

Hemostasis was attempted using a combination of nasal packing, anterior and posterior oropharyngeal tamponade and local application of hemostatic agents. The patient received multiple transfusions, including packed red blood cells (PRBCs), fresh frozen plasma (FFP), platelets, tranexamic acid and prothrombin complex concentrate. Given the concern for vasculitis-related hemorrhage, he was started on high-dose corticosteroid therapy (methylprednisolone 1g/day for three days).

Day 4-7: A contrast-enhanced CT scan was performed, revealing intracerebral hemorrhage (ICH) with surrounding edema, pleural effusion, pulmonary atelectasis and hypoplastic kidneys. Given the extent of bleeding, anticoagulation therapy for dialysis-related thrombotic risk was withheld. The patient's neurological status continued to decline, and neurosurgical consultation advised against surgical intervention due to poor overall prognosis.

Despite intensive medical therapy and collaborative input from rheumatology, nephrology and neurosurgery teams, the patient remained hemodynamically unstable with persistent lactic acidosis and worsening multiorgan failure. The repeated laboratory workup indicated disseminated intravascular coagulation (DIC) further complicating his clinical course.

Day 8: The patient suffered a sudden deterioration with refractory hypotension, bradycardia and pulseless electrical activity (PEA) arrest. Advanced cardiac life support (ACLS) was initiated, but despite prolonged resuscitative efforts return of spontaneous circulation (ROSC) was not achieved. The outcome was negative.

Discussion

FMF has been linked to various vasculitis disorders including Polyarteritis Nodosa (PAN)-like vasculitis. The presence of vasculitis in FMF patients significantly increases the risk of hemor-

rhagic complications, posing significant challenges in clinical management. PAN is a necrotizing vasculitis affecting small and medium sized arteries, leading to vascular inflammation, thrombosis and aneurysm formation. In FMF patients PAN-like vasculitis further exacerbates vascular fragility, making them highly susceptible to hemorrhagic events. The underlying inflammatory process disrupts the structural integrity of blood vessels by causing fibrinoid necrosis and fragmentation of the internal elastic lamina. This vascular damage leads to the formation of microaneurysms which are prone to rupture, resulting in both intracerebral and systemic hemorrhages (3).

Amyloid deposition exacerbates this process by infiltrating the vascular walls leading to progressive weakening of the tunica media and adventitia. This deposition compromises vascular compliance increasing the risk of spontaneous hemorrhage even in the absence of trauma or coagulopathy. In severe cases this can lead to life-threatening conditions, such as intracranial hemorrhage, massive gastrointestinal bleeding or diffuse alveolar hemorrhage.

Patients with FMF and PAN-like vasculitis may present with various hemorrhagic manifestations including epistaxis and mucosal bleeding that is persistent nasal and oropharyngeal hemorrhage often requiring aggressive hemostatic interventions. Gastrointestinal hemorrhage like amyloid-related vasculopathy can lead to gastrointestinal bleeding presenting as melena or hematemesis (4). Intracerebral hemorrhage is one of the most severe manifestations, intracranial bleeding occurs due to the rupture of amyloid-weakened vessels leading to neurological deterioration and high mortality rates. Retroperitoneal and perirenal hematomas can occur due to vascular rupture around the kidneys particularly in patients with amyloidosis related nephropathy.

The management of hemorrhagic complications in FMF patients with PAN-like vasculitis is particularly challenging due to the coexisting risks of thrombosis and bleeding. Hemostatic support in patients with active bleeding requires transfusions of packed red blood cells, fresh frozen plasma and platelets. The use of antifibrinolytic agents such as tranexamic acid should be considered but must be carefully monitored to avoid thrombotic complications (5). Immunosuppressive therapy like corticosteroids and immunomodulatory drugs are essential for controlling inflammation and preventing further vascular damage. However, high-dose corticosteroids can increase the risk of gastrointestinal bleeding, necessitating gastroprotective measures such as proton pump inhibitors (6). Dialysis considerations in FMF patients with ESRD undergoing hemodialysis are at heightened risk of uremic platelet dysfunction further exacerbating bleeding tendencies. The decision to use anticoagulation during dialysis should be individualized balancing the risks of thrombosis and hemorrhage (7). Multidisciplinary approach is essential to navigate the complex interplay of inflammation, vasculitis and hemorrhagic risks.

Hemorrhagic complications in FMF patients with PAN-like vasculitis are a significant cause of morbidity and mortality driven by vascular inflammation and amyloid deposition. The increased fragility of small and medium sized arteries predisposes these patients to bleeding events including intracranial hemorrhage and gastrointestinal bleeding (8). Given the complexity of these cases early recognition, careful hemostatic management and a multidisciplinary treatment strategy are paramount in improving patients' outcomes. Further research is needed to better understand the mechanisms linking FMF, PAN-like vasculitis and hemorrhagic risk to develop targeted therapies for these high-risk patients.

Renal involvement is the most severe long-term complication, which is due to systemic amyloidosis. FMF-associated amyloidosis leads to progressive renal dysfunction and ultimately

end-stage renal disease (ESRD) necessitating long-term dialysis (9). The presence of ESRD not only worsens patients' outcomes but also contributes to a higher risk of complications including bleeding tendencies due to uremic platelet dysfunction.

Amyloidosis in FMF results from the excessive production and deposition of serum amyloid A (SAA) protein in various organs particularly the kidneys. These deposits progressively damage the glomeruli, impairing renal filtration and leading to proteinuria, nephrotic syndrome and eventually ESRD. Once kidney function deteriorates beyond a critical threshold, patients require renal replacement therapy in the form of hemodialysis or peritoneal dialysis (10).

Patients with ESRD on long-term dialysis face a heightened risk of bleeding due to multiple factors, most notably uremic platelet dysfunction. Uremia is a consequence of renal failure. It alters platelet adhesion, aggregation and secretion, leading to an increased tendency for spontaneous bleeding. This is particularly concerning in FMF patients with concurrent vasculitis where vascular fragility and amyloid deposition already predispose them to hemorrhagic events (11).

Early diagnosis and colchicine therapy for preventing amyloidosis through early and consistent colchicine use, remains the most effective strategy to avoid ESRD. Optimized dialysis management by adjusting dialysis parameters and minimizing the use of anticoagulants during dialysis sessions can help reduce bleeding risk. Use of hemostatic agents in patients with recurrent bleeding, desmopressin (DDAVP) and transfusions may be considered to improve platelet function (12). Regular monitoring for bleeding tendencies with routine laboratory tests including platelet function tests and coagulation profiles, are essential for early detection and intervention.

The management of Familial Mediterranean Fever (FMF) with amyloidosis is complex and requires a collaborative approach among multiple medical specialties. Given the multiorgan involvement of FMF, optimal patient care necessitates coordination between rheumatologists, nephrologists, intensivists, hematologists and neurosurgeons. The complexity of chronic inflammation, renal impairment and vascular complications demands a well-structured multidisciplinary strategy to improve patient's outcome and mitigate complications (13).

FMF is a systemic autoinflammatory disorder, when complicated by amyloidosis significantly impacts multiple organ systems. The deposition of amyloid protein in the kidneys often leads to end-stage renal disease (ESRD) requiring close monitoring by nephrologists. Rheumatologists play a pivotal role in managing the underlying inflammatory process primarily through colchicine therapy which is the first-line treatment to prevent amyloidosis. However, in patients with severe or refractory disease additional immunosuppressive therapies such as corticosteroids are necessary. Intensivists oversee supportive care in cases involving sepsis, hemorrhagic complications or multi-organ failure. Hematologists contribute by managing bleeding disorders associated with uremic platelet dysfunction and amyloid-related vascular fragility, while neurosurgeons may be required in cases of intracranial hemorrhage due to amyloid angiopathy or vasculitis-associated complications.

Immunosuppressive therapy like corticosteroids and biologic agents, is often necessary in FMF patients with severe inflammatory manifestations or vasculitis. Their use must be carefully balanced against the increased risk of infections and hemorrhagic complications in patients undergoing dialysis. High dose of corticosteroids may provide significant anti-inflammatory benefits but can exacerbate the risk of sepsis in immunocompromised individuals. Biologic therapies targeting interleukin-1 (IL-1) and tumor necrosis factor (TNF) pathways offer promising results

in controlling inflammation but require vigilant monitoring for opportunistic infections (14).

Early diagnosis, regular monitoring of renal function and colchicine therapy are critical for preventing the progression of amyloidosis and improving long-term prognosis. Future research is necessary to refine therapeutic strategies and improve long-term outcomes for FMF patients with amyloidosis.

Conclusion

We present a rare case of FMF with amyloidosis, complicated by massive hemorrhage, intracerebral bleeding and ESRD. This case highlights the increased risk of vasculitis-associated hemorrhagic events in FMF patients especially those with PAN-like vasculitis. Amyloid deposition in vascular structures leads to fragility and rupture making the management of these patients exceptionally challenging. The relationship between FMF and systemic vasculitis requires further investigation to better understand underlying mechanisms and improve therapeutic strategies. Early colchicine therapy and close monitoring are paramount in reducing FMF complications and improving patients' outcomes.

References:

1. Ozdogan H, Ugurlu S. Familial Mediterranean Fever. Presse Med. 2019 Feb;48(1 Pt 2): e61-e76.
2. Siligato R, Gembillo G, Calabrese V, Conti G, Santoro D. Amyloidosis and Glomerular Diseases in Familial Mediterranean Fever. Medicina (Kaunas). 2021 Oct 1;57(10):1049. doi: 10.3390/medicina57101049.
3. Ozen S, Ben-Chetrit E, Bakkaloglu A,et al. Polyarteritis nodosa in patients with Familial Mediterranean Fever (FMF): a concomitant disease or a feature of FMF? Semin Arthritis Rheum. 2001 Feb;30(4):281-7. doi: 10.1053/sarh.2001.19958.
4. Abbara S, Grateau G, Ducharme-Bénard S, Saadoun D, Georgin-Lavialle S. Association of Vasculitis and Familial Mediterranean Fever. Front Immunol. 2019 Apr 12; 10:763. doi: 10.3389/fimmu.2019.00763.
5. Aksu K, Keser G. Coexistence of vasculitides with familial Mediterranean fever. Rheumatol Int. 2011 Oct;31(10):1263-74. doi: 10.1007/s00296-011-1840-z.
6. Kiraz, S., & Çolak, İ. (2013). The Role of Immunosuppressive Agents in the Treatment of Familial Mediterranean Fever. Current Drug Therapy, 8(1), 25-32. https://doi.org/10.2174/1574885521308010208.
7. Sapan, C. V., & Tüzün, S. (2015). Amyloidosis in Familial Mediterranean Fever. Nephrology Dialysis Transplantation, 30(6), 941-948. https://doi.org/10.1093/ndt/gfv060.
8. Karadag O, Telli TA, Sayinalp N, Ertenli I. P01-003 – Bleeding disorder in FMF. Pediatr Rheumatol Online J. 2013 Nov 8;11(Suppl 1): A7. doi: 10.1186/1546-0096-11-S1-A7.
9. Lachmann HJ. Clinical aspects of familial Mediterranean fever. Nephrol Dial Transplant. 2001.

10. Gül, A., & Özen, S. (2017). Clinical aspects of amyloidosis in familial Mediterranean fever: An overview. Rheumatology, 56(1), 52-58. https://doi.org/10.1093/rheumatology/kew462.

11. Vaziri, N. D., & Rad, N. S. (2012). Uremic platelet dysfunction in end-stage renal disease. Nephron Clinical Practice, 121(1-2), c90-c99. https://doi.org/10.1159/000335290

12. Oner A, Erdoğan O, Demircin G, Bülbül M, Memiş L. Efficacy of colchicine therapy in amyloid nephropathy of familial Mediterranean fever. Pediatr Nephrol. 2003 Jun;18(6):521-6. doi: 10.1007/s00467-003-1129-x. Epub 2003 Apr 16. PMID: 12698329.

13. Tunca, M., & Onen, F. (2009). The management of familial Mediterranean fever. Clinical and Experimental Rheumatology, 27(4), 609-616.

14. Ozen S, Bilginer Y. A clinical guide to autoinflammatory diseases: familial Mediterranean fever and next-of-kin. Nat Rev Rheumatol. 2014 Mar;10(3):135-47. doi: 10.1038/nrrheum.2013.174. Epub 2013 Nov 19. PMID: 24247370.

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Dag Stat. Mackinnon A. Available from :http//www.mhri.cdu.au/biostats.Accessed May 5th 2006.

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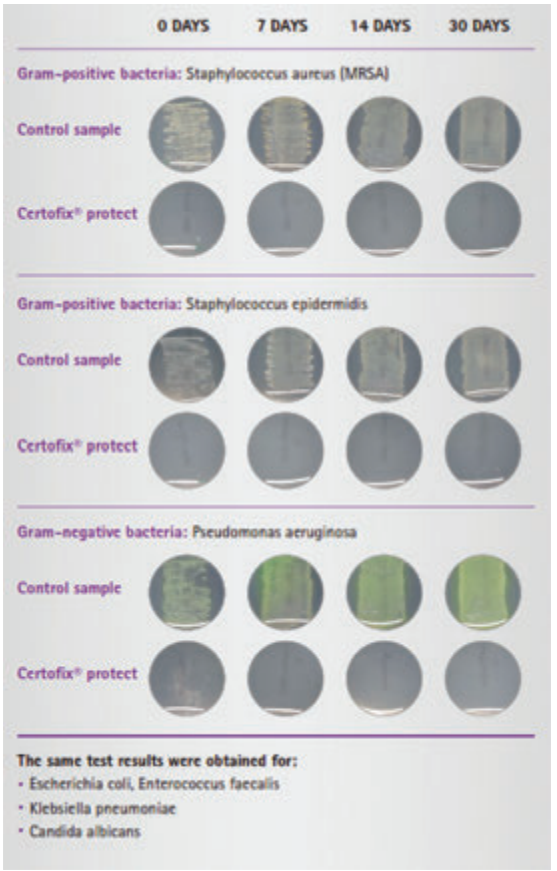

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