UNIVERSITATEA
TITU MAIORESCU

The 19th International Conference

EDUCATION AND CREATIVITY
FOR A KNOWLEDGE BASED SOCIETY

MEDICINE PROCEEDING BOOK

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PUBLISHER

Kürşat Çapraz

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e-ISBN:978-625-92035-0-8

9786259203508

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Publisher

Kürşat ÇAPRAZ, InTraders Academic Platform, Türkiye

DOI: https://doi.org/10.66793/titum19proceeding

Book Title

Universitatea Titu Maiorescu, The 19th International Conference, Education and creativity for a knowledge based society, Medicine Proceeding Book

Book Field: Medicine

e-ISBN: **978-625-92035-0-8**

Edition: First Edition, 08 June 2026, Babaeski, Kırklareli, Türkiye

Language: English

* Responsibility for content and originality of the text rests solely the author / the authors

Publisher

Kürşat ÇAPRAZ

InTraders Academic Platform, Kırklareli, Turkey

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International Conference **"**Education and creativity for a knowledge based society (19th edition)", Bucharest, Romania, November 20 - 22, 2025

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INTEGRATED ANTIVIRAL AND ANTIDIABETIC THERAPY IN CANCER PATIENTS WITH COVID-19: A RETROSPECTIVE CLINICAL STUDY

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DOI: https://doi.org/10.66793/titum19proceeding1

ABSTRACT

During the COVID-19 pandemic, surgical management of cancer patients was severely limited due to viral complications and systemic contraindications, resulting in increased mortality among oncologic patients. The overlap between cancer, diabetes mellitus, and COVID-19 infection has created a high-risk clinical profile, characterized by metabolic imbalance, inflammatory response, and multi-organ dysfunction. This study aimed to evaluate the therapeutic efficacy of integrated antiviral and antidiabetic regimens in improving biological stability and reducing perioperative risks in cancer patients infected with SARS-CoV-2.

A retrospective clinical analysis was conducted on 275 cancer patients hospitalized between 2020 and 2021 in three tertiary centers: the National Institute of Infectious Diseases "Matei Balş", the Central Military Hospital, and affiliated intensive care units. The study included patients with concomitant COVID-19 infection and diabetes or persistent hyperglycemia. Four treatment cohorts were analyzed: (1) Glargine U100 monotherapy; (2) Glargine U100 combined with oral antidiabetic agents; (3) Glargine U100 with Alpha-Lipoic Acid; and (4) Glargine U300 with Alpha-Lipoic Acid. Antiviral regimens included Molnupiravir, Anakinra, and Remdesivir, adjusted by disease severity. Clinical and biochemical markers (SpO_2, HbA1c, CRP, IL-1, TNF-α, D-dimer, lipid profile, renal and hepatic function) were monitored from admission to discharge.

Combined antiviral and antidiabetic therapy significantly reduced inflammatory and metabolic parameters, improving oxygen saturation and glycemic control. Molnupiravir demonstrated marked efficacy in reducing viral persistence and cytokine-mediated inflammation, with a 0.38% in-hospital mortality rate among diabetic cancer patients. Concomitant use of oral antidiabetic agents (Semaglutide, Empagliflozin, Vildagliptin, Gliquidone) optimized metabolic balance, decreased insulin resistance, and mitigated multi-organ complications. Insulin glargine regimens stabilized glycemic profiles and reduced ketoacidosis risk, facilitating preoperative optimization.

Integrated antiviral and antidiabetic therapy provides a feasible and effective strategy for managing cancer patients with COVID-19 infection, reducing viral load, metabolic derangements, and perioperative contraindications. The combined therapeutic approach improved survival and biological readiness for surgical intervention, supporting its incorporation into multidisciplinary oncologic care during viral pandemics.

KEYWORDS: *COVID-19, Cancer, Diabetes Mellitus, Antiviral Therapy, Antidiabetic Agents*

INTRODUCTION

The COVID-19 pandemic has profoundly disrupted the global healthcare system, significantly affecting the management and prognosis of oncologic patients (1,2). The emergence of the SARS-CoV-2 virus has introduced major clinical challenges, particularly in patients with pre-existing malignancies and metabolic disorders such as diabetes mellitus (3). During the early phases of the pandemic, a

substantial proportion of cancer patients experienced delayed or suspended surgical interventions due to the high risk of viral transmission, postoperative complications, and respiratory failure (4). The cumulative impact of these restrictions resulted in an alarming increase in morbidity and mortality among oncologic populations worldwide (5).

The association between cancer, diabetes mellitus, and COVID-19 infection represents a unique clinical triad that amplifies systemic inflammation, impairs immune response, and aggravates metabolic dysregulation (6). Hyperglycemia and insulin resistance have been identified as critical determinants of poor outcomes in COVID-19, increasing the likelihood of cytokine storm, thromboembolic events, and multiorgan dysfunction (7). In cancer patients, these processes further compromise the metabolic and inflammatory balance already altered by neoplastic progression and cytotoxic therapy. Therefore, the identification of therapeutic strategies capable of controlling both viral and metabolic pathways has become essential for improving the survival and surgical eligibility of these patients (8).

Antiviral drugs such as Molnupiravir, Anakinra, and Remdesivir have demonstrated efficacy in reducing viral replication and systemic inflammation (3,9,10), while modern antidiabetic therapies—including insulin analogs (Glargine U100/U300) and oral agents such as Semaglutide, Empagliflozin, Vildagliptin, and Gliquidone—offer improved glycemic control with additional cardiometabolic benefits (11–13). The integration of these therapeutic classes in patients with overlapping oncologic, infectious, and metabolic pathologies may mitigate the severity of COVID-19 infection and reduce contraindications to curative surgical interventions (14).

This retrospective study was designed to evaluate the clinical outcomes of integrated antiviral and antidiabetic therapy in cancer patients infected with COVID-19. By analyzing biochemical, metabolic, and inflammatory markers across different therapeutic regimens, the study aims to establish the efficacy of combined treatment strategies in restoring physiological stability, minimizing perioperative risk, and enabling timely surgical management in oncologic patients affected by the pandemic.

MATERIALS AND METHODS

This retrospective, multicenter clinical study was conducted between 2020 and 2021, during the peak of the COVID-19 pandemic, across three tertiary medical institutions in Bucharest, Romania: the National Institute of Infectious Diseases “Prof. Dr. Matei Balș”, the Central Military Emergency Hospital, and associated intensive care units. The study aimed to evaluate the outcomes of integrated antiviral and antidiabetic therapy in cancer patients co-infected with SARS-CoV-2, with or without pre-existing diabetes mellitus.

A total of 275 patients aged between 30 and 60 years were included. All subjects were diagnosed with various malignant neoplasms requiring surgical intervention according to standard oncologic protocols but whose operations were postponed due to active COVID-19 infection or severe metabolic and systemic contraindications. The main types of malignancies represented in the cohort were: ovarian (22%), prostate (22%), pulmonary (16%), breast (10%), renal (5%), thyroid (8%), and a smaller group of digestive and lymphatic cancers (approximately 12%). Each patient presented either confirmed diabetes mellitus or persistent hyperglycemia throughout the viral infection. A small control group of non–COVID-19 cancer patients was also analyzed for comparative purposes.

The antiviral regimen included early initiation of Molnupiravir in patients with mild to moderate COVID-19, particularly those with hyperglycemia or uncontrolled diabetes, followed—when clinically indicated—by combination therapy with Anakinra and Remdesivir to mitigate cytokine-mediated inflammatory responses. In severe cases with pulmonary involvement (“ground-glass” opacities) or systemic complications, oxygen therapy and anti-inflammatory agents such as Dexamethasone or Colchicine were added.

The antidiabetic management strategy involved both oral and insulin-based therapies, adjusted according to blood glucose dynamics, HbA1c, and inflammatory markers. Four treatment cohorts were defined:

1. Patients treated with Insulin Glargine U100 monotherapy;
2. Patients receiving Glargine U100 combined with oral antidiabetic drugs (OADs);
3. Patients treated with Glargine U100 plus Alpha-Lipoic Acid;

4. Patients treated with Glargine U300 plus Alpha-Lipoic Acid.

Additional oral therapies included Semaglutide (GLP-1 agonist), Empagliflozin (SGLT-2 inhibitor), Vildagliptin (DPP-4 inhibitor), and Gliquidone (sulfonylurea). These regimens were selected according to the metabolic profile, inflammatory state, and comorbidities of each patient.

Clinical, biochemical, and imaging data were retrospectively extracted from patient medical records. Laboratory evaluations were performed at hospital admission, during hospitalization, and at discharge, including: fasting and random glucose, HbA1c, lipid profile (HDL, LDL, triglycerides), inflammatory markers (CRP, IL-1, IL-6, TNF-α, PAI-1, D-dimer), renal and hepatic function (creatinine, transaminases, uric acid), and arterial oxygen saturation (SpO_2). Imaging investigations—primarily chest CT scans—were used to assess pulmonary involvement and treatment response.

Data were analyzed using descriptive and inferential statistical methods. Distribution normality was tested using the Kolmogorov–Smirnov test. Continuous variables were expressed as means ± standard deviation (SD) or medians with interquartile range (IQR), depending on data distribution. Comparisons between treatment groups were performed using Student's *t*-test or Mann–Whitney *U*-test for continuous variables, and chi-square test for categorical variables. Correlations between biochemical markers and clinical outcomes were assessed using Pearson or Spearman coefficients, as appropriate. A p-value <0.05 was considered statistically significant.

RESULTS

Clinical outcomes

A total of 275 oncologic patients with confirmed SARS-CoV-2 infection were included in the analysis. Baseline demographic and clinical characteristics are summarized in Table 1. Among these, 68% had pre-existing type 2 diabetes mellitus, while 32% developed new-onset hyperglycemia during hospitalization, suggesting either stress-related or viral-induced impairment of glucose metabolism. The mean age of the cohort was 52.4 ± 8.7 years, with a slight female predominance due to the inclusion of breast and ovarian cancer cases. Most patients presented with moderate to severe COVID-19 (72%), of whom 38% required oxygen supplementation and 12% required intensive care support.

Table 1. Baseline characteristics of the study population (n = 275)

Parameter	Total (n=275)	Male (%)	Female (%)	p-value
Age (years, mean ± SD)	52.4 ± 8.7	51.8 ± 9.1	53.0 ± 8.3	0.32
Diabetes mellitus (pre-existing)	187 (68%)	70	117	0.21
New-onset hyperglycemia	88 (32%)	38	50	—
Ovarian cancer	60 (22%)	–	60	—
Prostate cancer	60 (22%)	60	–	—
Pulmonary cancer	44 (16%)	28	16	—
Breast cancer	28 (10%)	–	28	—
Thyroid cancer	22 (8%)	5	17	—
Renal cancer	14 (5%)	9	5	—
Digestive / lymphatic / other	27 (10%)	12	15	—
COVID-19 severity (moderate/severe)	198 (72%)	—	—	—
Oxygen therapy required	105 (38%)	—	—	—
ICU admission	33 (12%)	—	—	—

Early antiviral therapy with Molnupiravir (800 mg/day) was initiated within the first 72 hours of admission in patients presenting with mild to moderate infection. As shown in Table 2, this regimen—alone or in combination with Anakinra and Remdesivir—led to significant clinical improvement, reflected by increasing oxygen saturation (mean $SpO_2 > 95\%$) and shorter hospitalization times (mean 7.2 ± 3.4 days). Patients receiving combined antiviral and anti-inflammatory therapy exhibited a more pronounced reduction of fever, dyspnea, and fatigue compared with those receiving standard supportive treatment.

Table 2. Therapeutic cohorts and pharmacologic regimens

Treatment Group	No. of Patients	Antiviral Regimen	Antidiabetic Therapy	Additional Agents	Clinical Outcome Summary
Group 1	160	Molnupiravir ± Anakinra	Glargine U100	—	Improved glycemia, ↓ CRP, ↑ SpO_2
Group 2	61	Molnupiravir ± Anakinra	Glargine U100 + OADs	Alpha-Lipoic Acid (optional)	Better glycemic stability and lipid profile
Group 3	30	Molnupiravir + Anakinra	Glargine U100 + Alpha-Lipoic Acid	—	↓ D-dimer, ↓ IL-1, improved renal markers
Group 4	24	Molnupiravir ± Remdesivir	Glargine U300 + Alpha-Lipoic Acid	—	Variable glycemic control, ↑ Ferritin, D-dimer

Metabolic and inflammatory response

Biochemical and inflammatory marker dynamics are presented in Table 3. The integration of antiviral and antidiabetic therapy resulted in a statistically significant decline in fasting glucose (−33.8%), HbA1c (−20.2%), and inflammatory parameters such as CRP (−73.8%), IL-6 (−60.4%), TNF-α (−58.3%), and D-dimer (−57.1%) ($p < 0.01$). Simultaneously, a favorable improvement of the lipid profile was observed, with an increase in HDL (+36.1%) and a decrease in LDL (−17.6%).

Table 3. Laboratory parameters before and after integrated therapy

Biomarker	Baseline (mean ± SD)	Discharge (mean ± SD)	% Change	p-value
Fasting glucose (mg/dL)	198 ± 47	131 ± 28	–33.8%	<0.001
HbA1c (%)	8.9 ± 1.2	7.1 ± 0.9	–20.2%	<0.001
CRP (mg/L)	84 ± 30	22 ± 9	–73.8%	<0.001
IL-6 (pg/mL)	48 ± 20	19 ± 8	–60.4%	<0.01
TNF-α (pg/mL)	36 ± 11	15 ± 6	–58.3%	<0.01
D-dimer (µg/mL)	2.1 ± 0.8	0.9 ± 0.4	–57.1%	<0.001
HDL (mg/dL)	36 ± 9	49 ± 10	+36.1%	<0.05
LDL (mg/dL)	142 ± 27	117 ± 23	–17.6%	<0.05
SpO_2 (%)	90.3 ± 4.1	96.2 ± 2.3	+6.5%	<0.001
Creatinine (mg/dL)	1.8 ± 0.6	1.2 ± 0.3	–33.3%	<0.01

The therapeutic regimens based on Insulin Glargine U100, especially when combined with Alpha-Lipoic Acid, provided superior metabolic stability and renal protection compared to Glargine U300 formulations. This combination achieved a consistent reduction in serum creatinine (−33.3%) and

improved oxygenation (SpO_2 96.2 ± 2.3%). Conversely, Glargine U300 regimens showed greater variability in dosing and were associated with higher ferritin and D-dimer levels, indicating less effective inflammatory control.

Among oral antidiabetic agents, Semaglutide and Empagliflozin demonstrated the most consistent metabolic benefits, reducing BMI and insulin resistance while improving cardiometabolic parameters. Vildagliptin normalized HDL cholesterol and 25-OH vitamin D levels in prostate cancer patients, whereas Gliquidone, either alone or in combination with Molnupiravir, effectively stabilized glycemia and prevented escalation to high-dose insulin therapy, particularly in patients with drug-induced or secondary diabetes.

Mortality and surgical eligibility

Clinical outcomes and mortality rates are detailed in Table 4. The overall in-hospital mortality among patients treated with Molnupiravir-based regimens was 0.38%, limited to cases with advanced thyroid malignancy and severe metabolic decompensation. Compared with the small control group lacking integrated therapy, the Molnupiravir cohort exhibited a significantly lower ICU transfer rate (12% vs 28%, $p < 0.05$), shorter hospitalization, and a higher rate of surgical readiness at discharge (72% vs 39%, $p < 0.01$).

Patients who achieved glycemic stability and normalization of inflammatory biomarkers were subsequently re-evaluated for oncologic surgery. In these cases, the integrated antiviral and antidiabetic strategy restored metabolic equilibrium, reduced perioperative contraindications, and improved postoperative eligibility. Overall, the combined therapeutic approach not only minimized viral and inflammatory burden but also optimized the biological conditions necessary for definitive cancer treatment during the pandemic period.

Table 4. Clinical outcomes and mortality rates

Clinical Parameter	Molnupiravir-based (n=275)	Without integrated therapy (n=30)	p-value
Mean hospital stay (days)	7.2 ± 3.4	11.6 ± 4.7	<0.01
Oxygen saturation (SpO_2 >95%) at discharge	91%	67%	<0.01
ICU transfer rate	12%	28%	<0.05
In-hospital mortality	0.38%	6.7%	<0.001
Recurrent infection within 30 days	4%	15%	<0.05
Readiness for surgical reevaluation	72%	39%	<0.01

DISCUSSION

The present study demonstrates that integrated antiviral and antidiabetic therapy significantly improves both metabolic and inflammatory control in oncologic patients affected by COVID-19 infection (9,10). The findings provide strong evidence that a dual pharmacologic strategy targeting viral replication and glucose homeostasis is critical for mitigating disease severity, reducing perioperative contraindications, and improving survival in this highly vulnerable population (11).

COVID-19 infection exerts a multifactorial impact on glucose metabolism, mediated by direct pancreatic β-cell injury, cytokine-driven insulin resistance, and corticosteroid exposure during treatment (12). In oncologic patients, these mechanisms are compounded by the systemic inflammatory milieu characteristic of malignancy and by metabolic derangements secondary to cachexia and treatment-induced stress (13). The high prevalence of hyperglycemia (32%) and pre-existing diabetes mellitus (68%) observed in this cohort supports previous reports describing diabetes as a major determinant of poor COVID-19 outcomes (14). Several studies have indicated that uncontrolled glycemia correlates

with increased interleukin (IL-6, IL-1β, TNF-α) expression, endothelial dysfunction, and thrombogenic risk, which together amplify the systemic inflammatory response and promote multiorgan injury (15,16).

The therapeutic results obtained with Molnupiravir confirm its efficacy as a first-line antiviral agent in cancer patients, not only in reducing viral load but also in attenuating the proinflammatory cytokine cascade (17). When combined with Anakinra, a selective IL-1 receptor antagonist, and Remdesivir, Molnupiravir contributed to the downregulation of key inflammatory mediators and the prevention of cytokine storm, as reflected by the significant reduction of CRP, IL-6, and TNF-α levels observed in our analysis (Table 3). These findings align with international evidence suggesting that early initiation of Molnupiravir in immunocompromised hosts is associated with a lower risk of hospitalization and mortality (18).

Similarly, the inclusion of targeted antidiabetic agents enhanced the metabolic resilience of these patients. Insulin Glargine U100, used as basal insulin, proved superior to U300 formulations in maintaining glycemic stability, minimizing ketoacidosis, and improving oxygen saturation, particularly when combined with Alpha-Lipoic Acid, an antioxidant with demonstrated effects on insulin sensitivity and endothelial protection (19). Among oral agents, Semaglutide and Empagliflozin were notable for their pleiotropic effects—improving lipid metabolism, reducing BMI, and enhancing cardiovascular safety. Vildagliptin and Gliquidone additionally contributed to glycemic control without aggravating hepatic or renal stress, an essential factor for maintaining surgical candidacy (20). These data corroborate recent findings from international cohorts emphasizing the role of SGLT2 inhibitors and GLP-1 receptor agonists in improving outcomes among diabetic patients with COVID-19.

An important observation of this study is the interdependence between inflammation, oxygenation, and glycemic control (14,15). Patients exhibiting a rapid decrease in inflammatory markers (CRP, IL-6, D-dimer) achieved faster normalization of SpO_2 and glycemia, reflecting a synergistic relationship between antiviral suppression, metabolic regulation, and oxygen delivery (17,18). Conversely, patients with persistently elevated inflammatory biomarkers showed delayed recovery and required prolonged hospitalization. This reinforces the concept that COVID-19 severity in diabetic cancer patients is largely driven by the interplay between viral pathogenesis and metabolic dysregulation (19).

The mortality rate observed in this cohort (0.38%) is remarkably lower than those reported in international literature for comparable populations (11,13), where rates of 5–20% are commonly described. This outcome likely reflects the early therapeutic intervention, multimodal drug regimen, and individualized titration of insulin and antiviral agents. Moreover, the integrated approach improved not only short-term outcomes but also preoperative readiness, as 72% of patients were deemed suitable for surgical re-evaluation after stabilization (9,16). This aspect is of particular clinical relevance, as surgical delay due to COVID-19 infection was among the leading causes of excess mortality in oncologic care during the pandemic (10,11).

From a pathophysiological standpoint, this study supports the emerging hypothesis that COVID-19 acts as both a viral and metabolic disease, in which insulin resistance, endothelial inflammation, and coagulopathy are interlinked (12,19,20). By simultaneously addressing viral replication and metabolic control, the therapeutic strategy employed in this cohort effectively interrupted this vicious cycle. The improvement in lipid and glucose metabolism, reduction in cytokine load, and normalization of renal and hepatic function markers suggest that combined therapy may exert a systemic modulatory effect, restoring homeostasis even in patients with multiple comorbidities.

Nevertheless, the study presents inherent limitations, including its retrospective design, lack of randomized control, and heterogeneity of cancer types and stages (14,15). Furthermore, while biochemical markers strongly indicate therapeutic benefit, long-term oncologic outcomes—such as recurrence rates and postoperative recovery—require prospective validation. Future multicenter prospective studies are warranted to confirm these findings, optimize treatment algorithms, and determine the most effective antiviral–antidiabetic combinations for cancer patients with concurrent COVID-19 infection (16,17).

In summary, the results of this investigation demonstrate that integrated antiviral and antidiabetic therapy constitutes a feasible and effective clinical strategy for managing cancer patients infected with SARS-CoV-2 (9,10,20). This multimodal approach mitigates the inflammatory and

metabolic burden, enhances survival, and facilitates timely surgical intervention, thereby providing a model for multidisciplinary management in complex comorbid conditions during infectious outbreaks (12,13).

CONCLUSIONS

This retrospective study highlights the clinical efficacy and safety of an integrated antiviral and antidiabetic therapeutic approach in cancer patients infected with COVID-19. The combined use of Molnupiravir-based antiviral therapy and individualized glucose-lowering regimens significantly reduced viral persistence, systemic inflammation, and metabolic dysregulation, leading to improved oxygenation and lower mortality.

Insulin Glargine, together with modern oral agents such as Semaglutide, Empagliflozin, Vildagliptin, and Gliquidone, proved to be effective in achieving glycemic stability and restoring metabolic homeostasis, while Alpha-Lipoic Acid supplementation enhanced renal and hepatic protection.

The therapeutic synergy between antiviral and antidiabetic agents not only minimized cytokine-driven complications but also facilitated biological readiness for surgical intervention in oncologic patients previously deemed inoperable.

Overall, these findings suggest that multimodal therapy targeting both viral and metabolic pathways can substantially improve short-term survival and surgical feasibility in cancer patients with COVID-19, providing a viable model for future integrated treatment protocols in complex comorbid populations.

CONFLICT OF INTEREST:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ETHICAL APPROVAL:

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Ethics Committee of the Carol Davila Central Emergency Military Hospital, Bucharest, Romania (protocol code 12009/17.09.2021).

CONSENT TO PARTICIPATE:

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

FUNDING:

This research received no external funding.

REFERENCES:

1. Litz BL, Bojko P, Smith EA. Integrated management of metabolic and viral comorbidities in oncologic patients during the COVID-19 pandemic. J Clin Med. 2023;12(4):1012–1023.
2. Holman N, Knighton P, Kar P, et al. Risk factors for COVID-19–related mortality in people with type 1 and type 2 diabetes in England: a population-based cohort study. Lancet Diabetes Endocrinol. 2020;8(10):823–833.
3. Jayk Bernal A, Gomes da Silva MM, Musungaie DB, et al. Molnupiravir for oral treatment of COVID-19 in nonhospitalized patients. N Engl J Med. 2022;386(6):509–520.
4. Cavalli G, Larcher A, De Luca G, et al. Interleukin-1 blockade with anakinra in COVID-19 patients: a prospective cohort study. Lancet Rheumatol. 2021;3(10):e633–e641.
5. Drucker DJ. Diabetes, obesity, metabolism, and SARS-CoV-2 infection: the end of the beginning. Cell Metab. 2021;33(3):479–498.
6. Scheen AJ. SGLT2 inhibitors and GLP-1 receptor agonists for the treatment of type 2 diabetes: major cardiovascular and renal benefits. Diabetes Res Clin Pract. 2023;198:110231.
7. Singh AK, Singh R. Hyperglycemia without diabetes and new-onset diabetes are both associated with poorer outcomes in COVID-19. Diabetes Res Clin Pract. 2020;167:108382.

8. Korytkowski M, Antinori-Lent K, Drincic A, et al. A pragmatic approach to inpatient diabetes management during the COVID-19 pandemic. J Clin Endocrinol Metab. 2020;105(9):3076–3087.
9. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 outbreak in China. JAMA. 2020;323(13):1239–1242.
10. Grajek S, Królicka AL, Mierzejewski P. COVID-19, diabetes and cancer: a triad of increased vulnerability. Front Oncol. 2022;12:925674.
11. Comparing Molnupiravir to Nirmatrelvir/Ritonavir (Paxlovid) in the Treatment of Mild-to-Moderate COVID-19 in Immunocompromised Cancer Patients — Haddad AJ, Hachem RY, Moussa M, Jiang Y, Dagher HR, Chaftari P, Chaftari A-M, Raad II. Cancers. 2024;16(5):1055.
12. COVID-19 Outcomes and Diabetes Mellitus — Akinosoglou K et al. Microorganisms. 2023;11(6):1416.
13. Outcomes of Cancer Patients Affected by COVID-19 in the United States: A Cohort Study — El Mahmoud A et al. PMC. 2024.
14. Risk Factors for COVID-19-Related Hospitalization and Death in Patients with Active Cancer Receiving Treatment — Rini BI et al. JAMA Oncology. 2025.
15. Adverse Outcomes in COVID-19 and Diabetes — Izzi-Engbeaya C et al. BMJ Open Diabetes Research & Care. 2021;9:e001858.
16. Efficacy and Safety of Molnupiravir Treatment for COVID-19 — Tian F et al. PMC. 2023.
17. Linking COVID-19 and Cancer: Underlying Mechanism — Tyagi S et al. Cancer Treatment Reviews. 2025 (in press).
18. Effectiveness and Safety of Molnupiravir in the Intended-Use Population — Ahmad WA et al. Clinical Microbiology and Infection. 2024.
19. Long Term Health Outcomes in People with Diabetes 12 Months after COVID-19 — Gharibzadeh S et al. eClinicalMedicine. 2025.
20. Clinical Outcomes among COVID-19 patients initiated on various antivirals: a systematic review and meta-analysis — Larsen CS et al. Expert Review of Anti-Infective Therapy. 2025.

LATE-ONSET LEFT VENTRICULAR OUTFLOW TRACT OBSTRUCTION FOLLOWING MITRAL VALVE REPLACEMENT IN HYPERTROPHIC CARDIOMYOPATHY: DIAGNOSTIC AND THERAPEUTIC CHALLENGES

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DOI: https://doi.org/10.66793/titum19proceeding2

Abstract

Hypertrophic obstructive cardiomyopathy is a genetic disease of the myocardium, characterized by symptoms of progressive heart failure, secondary to left ventricular outflow tract (LVOT) obstruction. Implantation of a prosthesis in the mitral position to address the valvular pathology will not improve the patient's clinical condition, who will continue to be symptomatic due to persistent intracardiac obstruction. Diagnosing LVOTO in patients with an implanted mitral prosthesis is often challenging with standard transthoracic echocardiography, but transesophageal echocardiography and cardiac CT imaging provide valuable diagnostic accuracy. Surgical intervention using a transverse aortotomy proved effective in this case, allowing direct visualization and resolution of obstruction without replacing the mitral prosthesis, consequently reducing operative time and avoiding unnecessary procedural risks.

CASE PRESENTATION

A 50 - year - old woman diagnosed with hypertrophic obstructive cardiomyopathy, history of mechanical prosthesis implantation in mitral position for severe regurgitation (2017), with recurrent episodes of atrial fibrillation, is admitted to our cardio-vascular unit in order to undergo septal myectomy . The patient was symptomatic by dyspnea on small exertion and constrictive chest pain, associating phenomena of congestive heart failure (NYHA class III heart failure). Our patient had also a history of persistent atrial fibrillation with radiofrequency ablation in 2023.

Physical examination on admission revealed a patient with good general condition , BMI 29 kg/ m2 , hemodynamically and respiratory stable. Cardiac auscultation noted well audible prosthetic clicks in the mitral area, third degree systolic murmur in the aortic area, mild ankle edema and no pulmonary crackles.

Current outpatient treatment included: Bisoprolol 5 mg/ day, Acenocumarol, Atorvastatin 20 mg/ day, Euthyrox 50 mcg/ day, Furosemide 40 mg/ day .

INITIAL WORK-UP

The electrocardiogram on admission revealed sinus rhythm, ventricular pacing rhythm , QRS duration 168 msec, PR interval 180 msec , QT duration 510 msec , major left bundle branch block (LBBB) .

Transthoracic preoperative X-ray revealed : transverse heart diameter at the upper normal limit, suture sternal cerclage wires, mechanic prosthesis in mitral position . No acute pleuro-pulmonary injuries were found.

Transthoracic preoperative echocardiography revealed non-dilated left ventricle (LV), with asymmetric severe hypertrophy, predominantly involving the interventricular septum [Fig 1], which had a maximum thickness of 30 mm, no wall motion abnormalities, but with apical rocking secondary to LBBB, normal LV systolic function . Right ventricle (RV) had normal function based on surrogate parameters. The mitral prosthesis had a normal function, with normal transprothetic gradients and an excess of pannus extending towards the LVOT, with subvalvular flow aortic acceleration[Fig 2]. Significant reduction of the LVOT diameter of only 11 mm was noted[Fig 1, 3]. Maximum resting LVOT velocity was 5.91 m/s, peak gradient of 146 mmHg and mean gradient 56 mmHg (at rest, no provocative manoeuvres) [Fig3] . Aortic valve was tricuspid and competent. Possible pulmonary hypertension was suspected and there was no pericardial effusion.

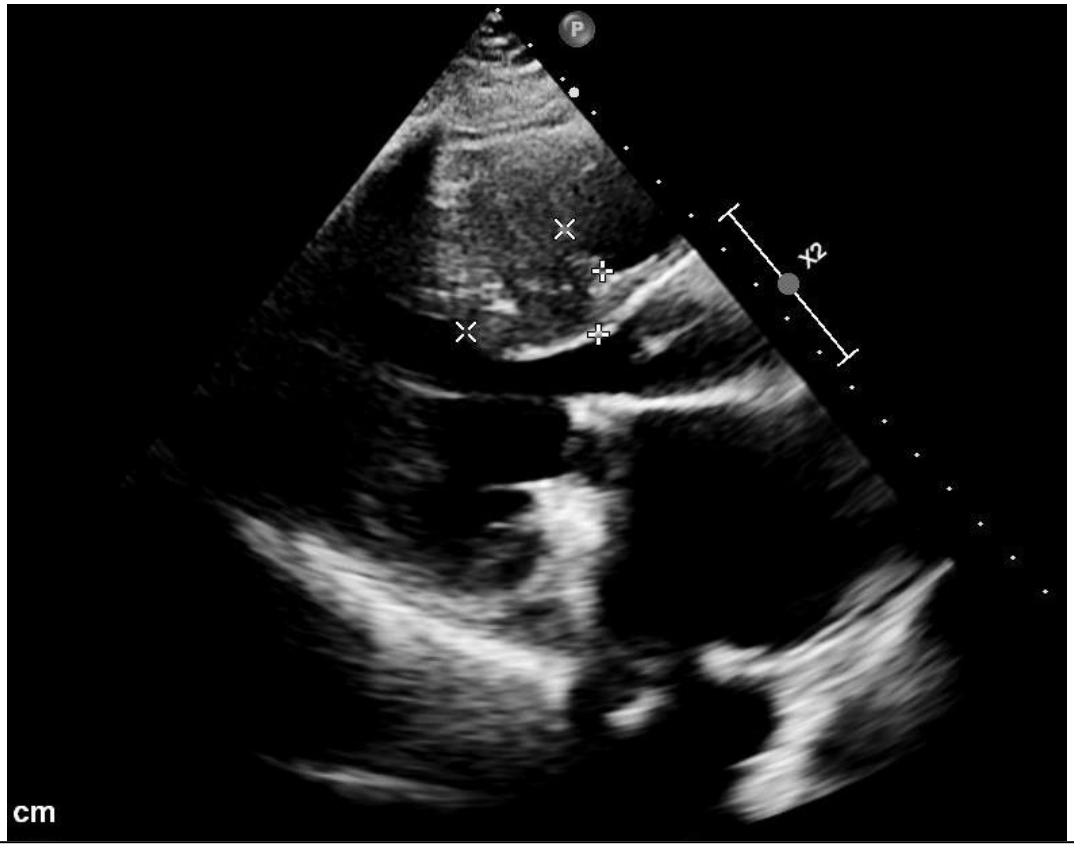

Fig 1. Severe interventricular septum hypertrophy with narrowing of the LVOT diameter

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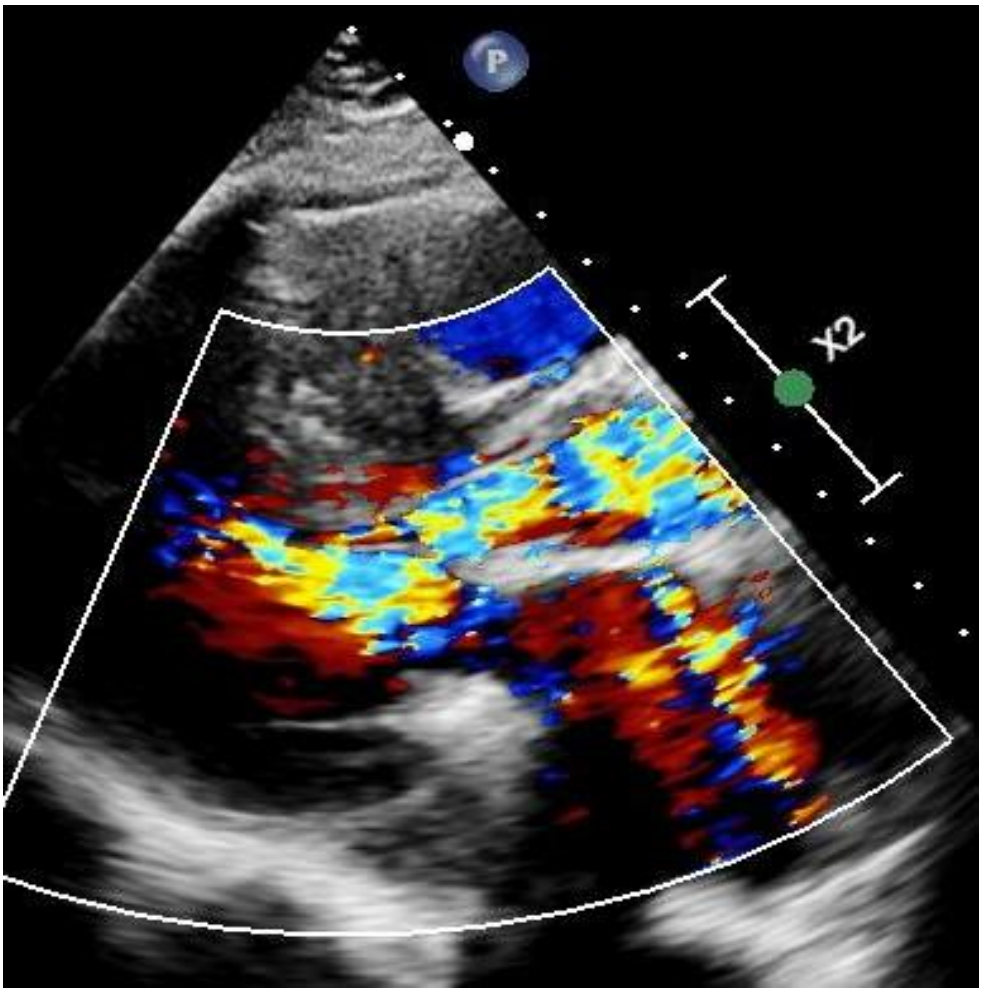

Fig 2: Color Flow Doppler shows LVOT flow turbulence due to LVOT obstruction

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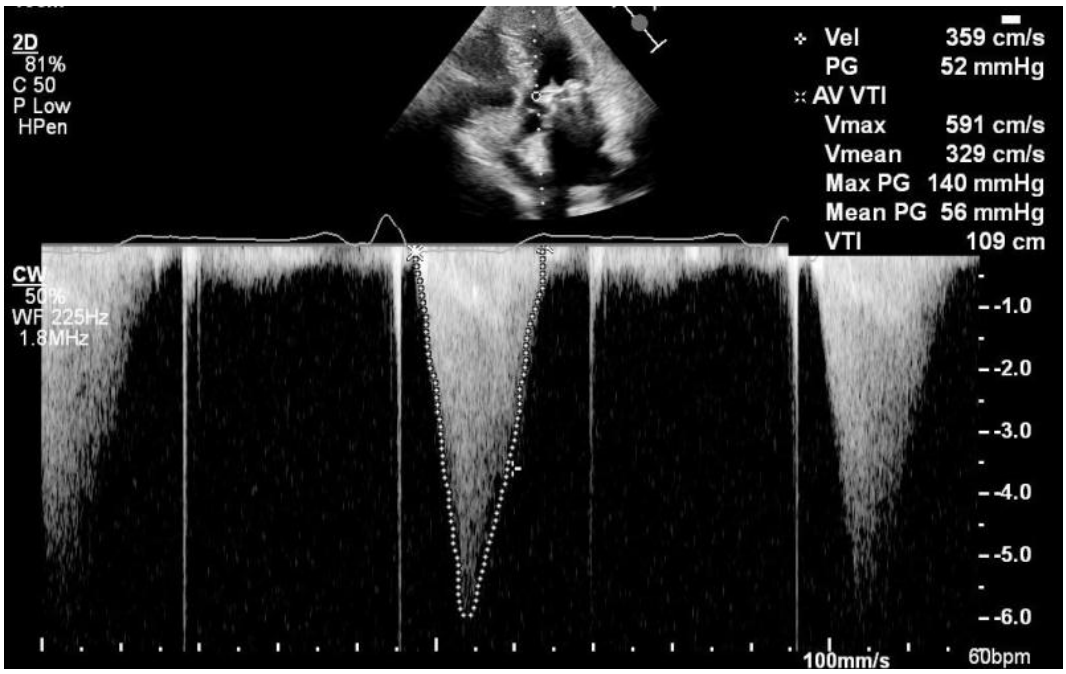

Fig 3. Continous flow Doppler: Peak and mean gradients at the LVOT level

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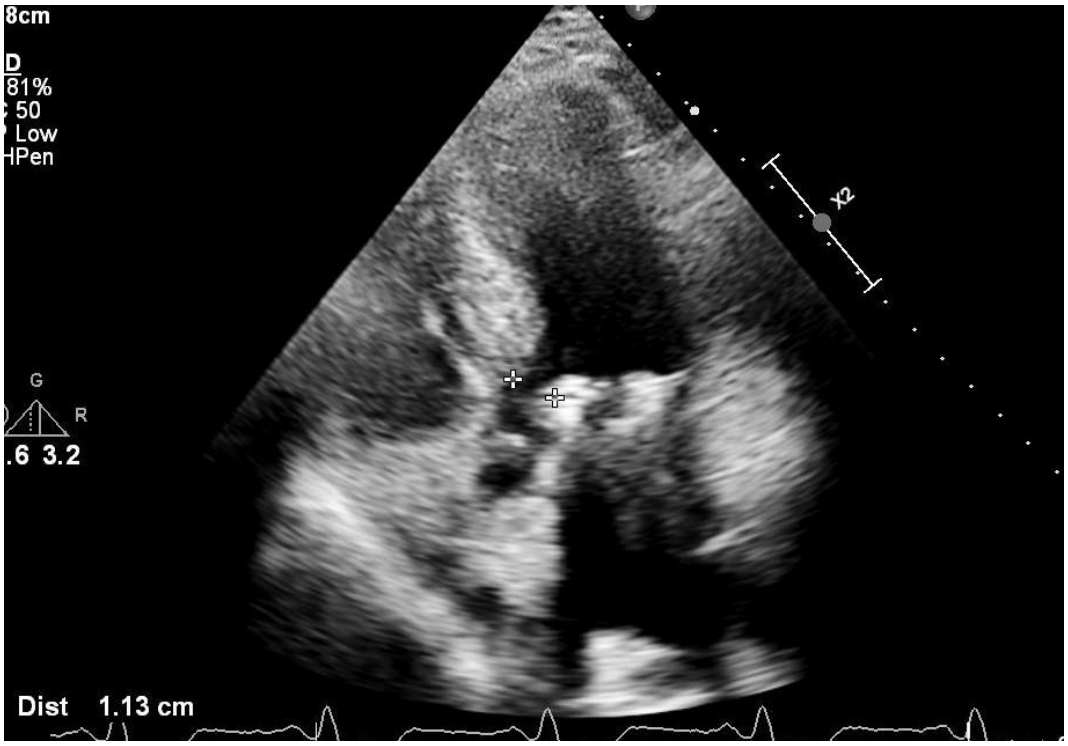

Fig 4. LVOT reduced diameter of only 11mm

Coronary angiography did not highlighted significant epicardial coronary stenoses. Respiratory tests (spirometry) were within normal limits. Abdominal ultrasound revealed mild hepatic steatosis and uncomplicated left renal cyst .

DIAGNOSIS AND MANAGEMENT

Based on clinical and paraclinical data, the working diagnoses were:

Severe obstructive hypertrophic cardiomyopathy with significant dynamic obstruction of the left ventricular ejection tract. NYHA class III heart failure. Bidisk mitral valve prosthesis implanted in 2017 with normal function .Radiofrequency ablation for persistent atrial fibrillation (2023). Dyslipidemia. Mild hepatic steatosis. Hypothyroidism on replacement therapy. Overweight .

After case review, the Heart Team recommended surgical septal myectomy , considering persistent symptoms and severe obstruction at the LVOT level, despite maximal medical treatment (class IB indication according to the ESC Guidelines).

Preoperative transesophageal echocardiography evaluated the mechanism of obstruction in the LVOT and also revealed flow acceleration and significant high gradients in the LVOT. Mitral valve disks had normal mobility and no intra or paraprosthetic leaks were recorded[Fig 5].

3D transesophageal echocardiography provided additional diagnosis, clearly demonstrating severe LVOT obstruction secondary to septal hypertrophy [Fig 6, 7] .

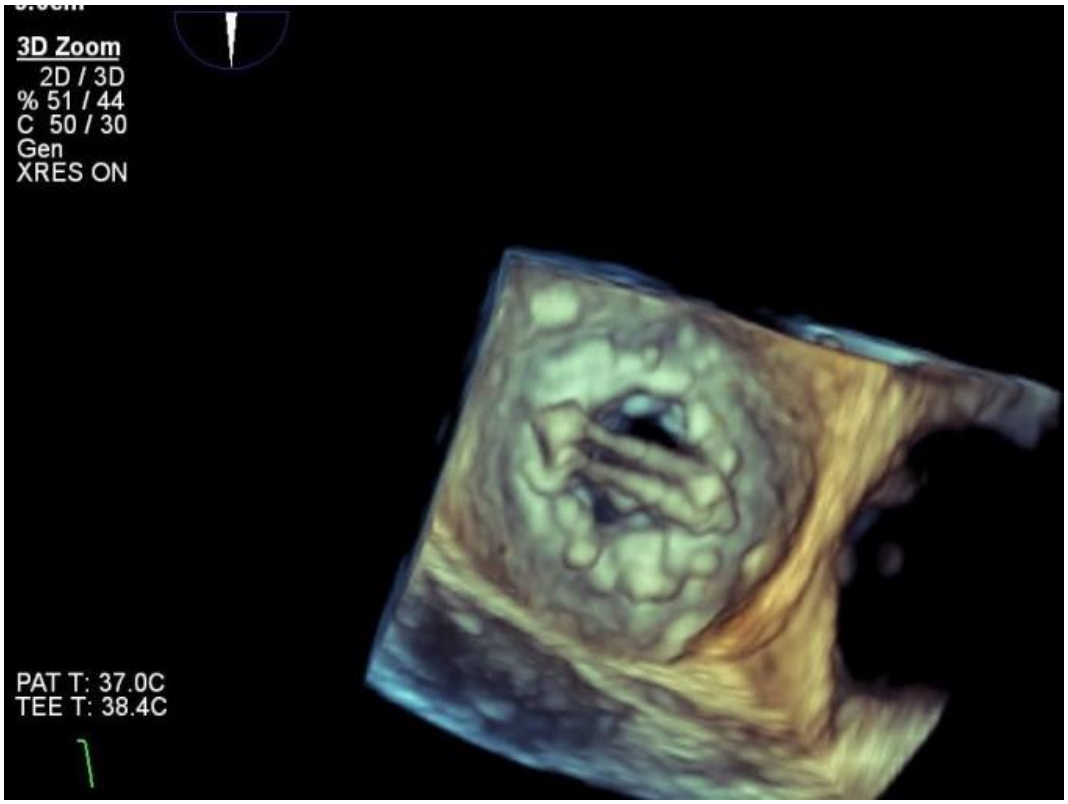

Fig 5:3D TEE: mitral prosthesis (atrial perspective): normal mobility of the disks which are full opened in diastole

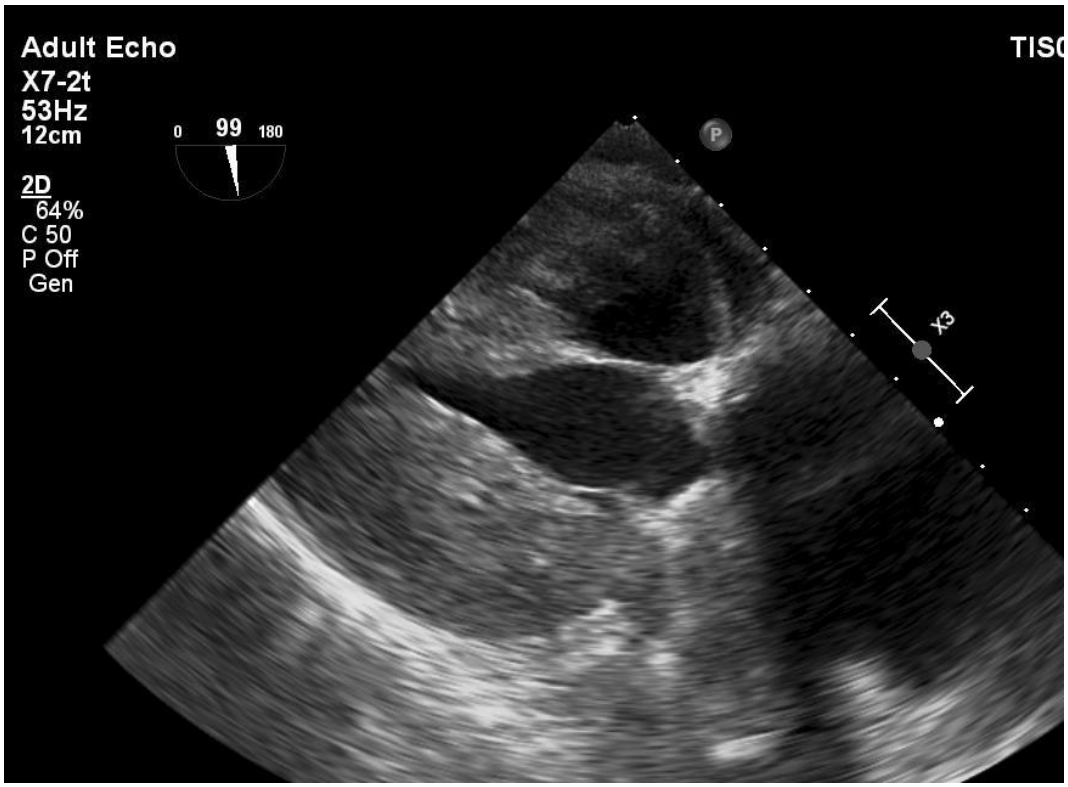

Fig 6: TEE Transgastric long axis view of the left ventricle demonstrates severe hypertrophy of the walls , small LVcavity

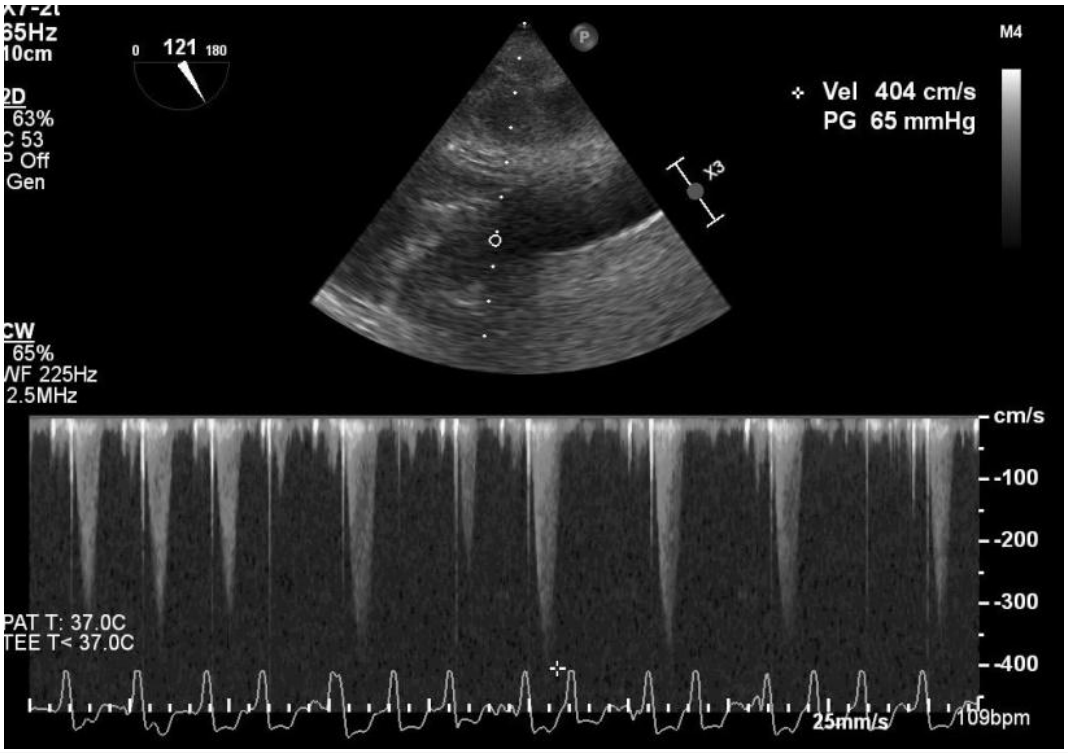

Fig 7. TEE deep transgastric view:continuous flow Doppler shows peak velocity of the flow in the LVOT of 4 m/s

Cardiac CT scan confirmed LVOT narrowing in diastole and turbulent flow at this level in systole [Fig 8,9].

The surgical intervention was performed via median sternotomy under extracorporeal circulation support. Following transverse aortotomy , intraoperative inspection revealed extensive endocardial circumferential fibrosis at the level of the LVOT, involving the anterior base of the mitral prosthesis , which has been anteriorly retracted by fibrotic pannus, also contributing to obstruction at this level. Resection of the fibrotic areas was performed, together with anterior septal myectomy, with LVOT enlargement .

Postoperatively transesophageal echocardiography demonstrated reduction of dynamic gradients in the LVOT, normal functioning mitral valve prosthesis, normal biventricular contractility and laminar flow in the LVOT[Fig 10, 11]

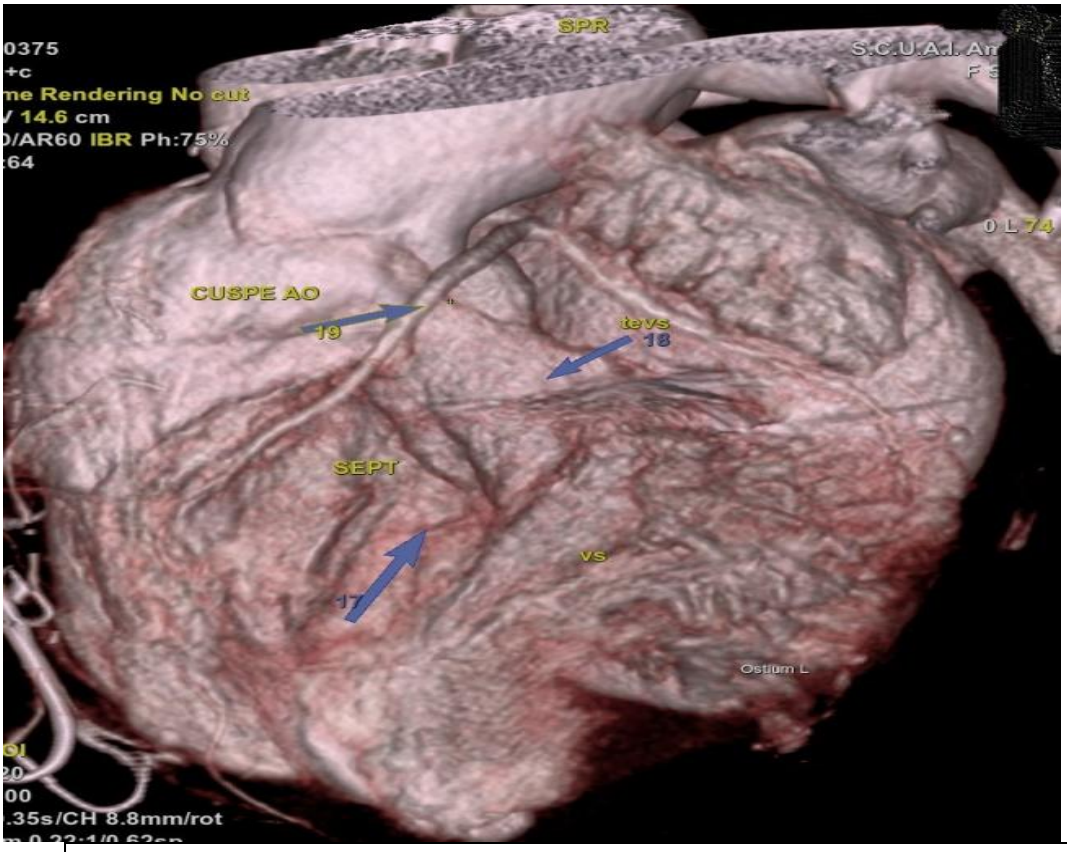

Fig 8: Cardiac CT confirms severe LVOT narrowing due to septal hypertrophy

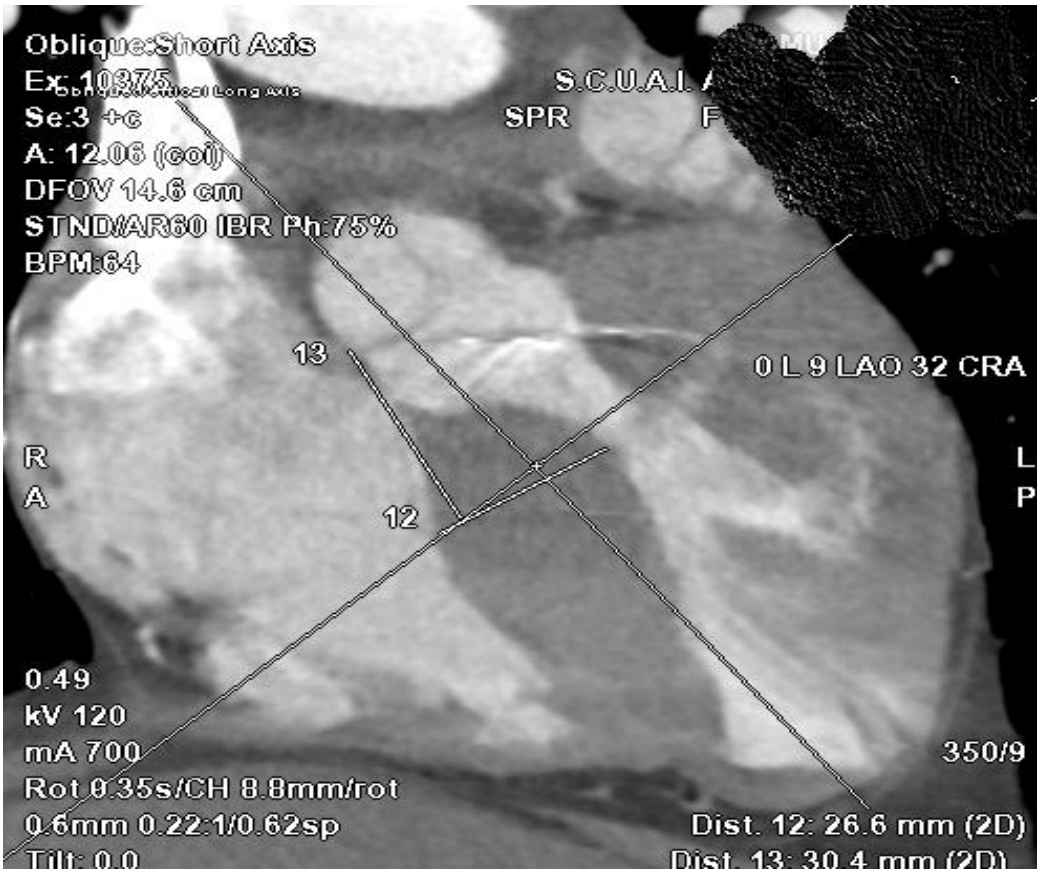

Fig. 9 .Cardiac CT showing severe septal hypertrophy, narrowed LVOT and small LV cavity

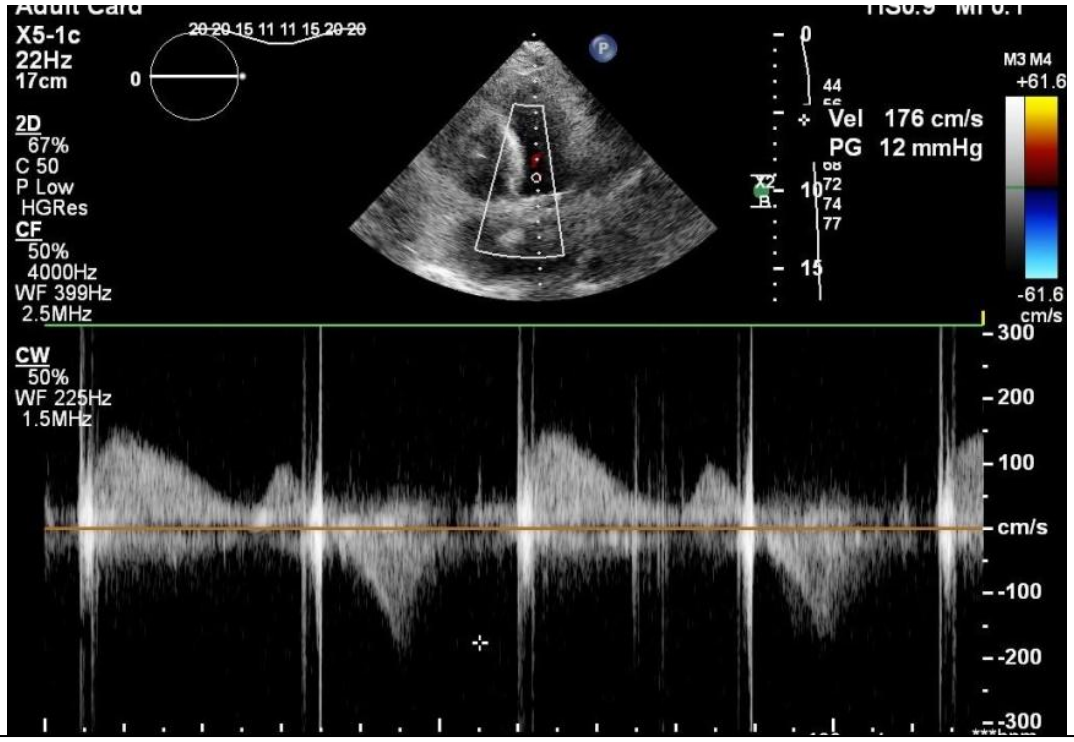

Fig 10: Transthoracic echocardiography 5 C view: postoperative peak flow velocity reduced to 1.7 m/s

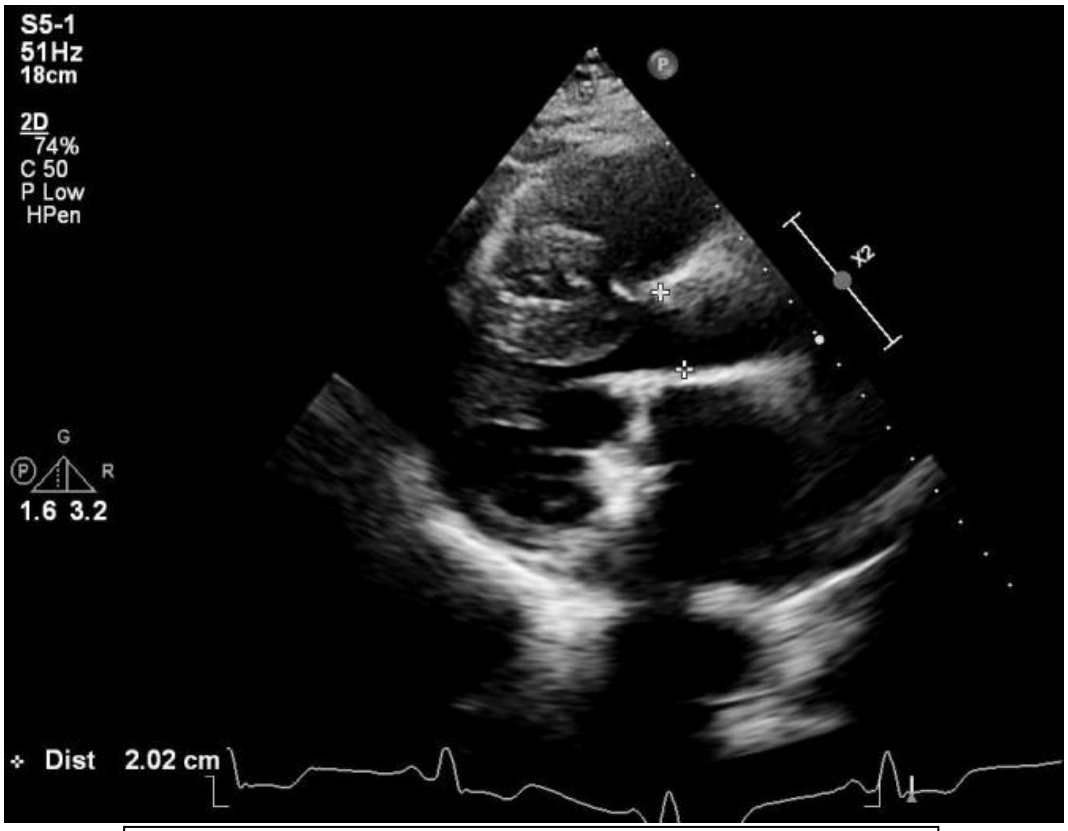

Fig 11. Postoperative enlargement of the LVOT (20 mm diameter)

FOLLOW-UP

In the ICU, our patient presented favorable postoperative evolution , afebrile, hemodynamically and respiratory stable after extubation, with no liver and renal dysfunction and she was transferred on Cardiovascular Surgery Department on postoperative day 4 . Clinical improvement continued; she was respiratory stable, with normal diuresis, normal liver and kidney function and with healing surgical wounds. During cardiac ward stay, the patient underwent respiratory and cardiac rehabilitation. Given the favorable paraclinical and clinical evolution , the patient was discharged on the postoperative day 6 with the following ambulatory medication : Acenocumarol , Bisoprolol 7.5 mg/ day , Amiodarone 100 mg/ day , Amlodipine 5 mg/ day , Torasemide 50 mg/ day , Atorvastatine 20 mg/ day , SGLT2 inhibitor, Euthyrox 50 mcg/ day and bronchodilatator therapy. She was recommended maintaining a Mediterranean diet, low-sodium, low-fat diet and advised to enroll in a cardiac rehabilitation program . In case of dental or surgical bleeding interventions it was recommended endocarditis prophylaxis.

At the 6- month reevaluation, our patient was in good general condition, without clinical signs of left or right heart failure, normal biventricular systolic function, normal valvular function, possible pulmonary hypertension and no pericardial fluid. Echocardiography revealed no subaortic obstruction and a mid -ventricular gradient of 25-39 mmHg at rest.

DISCUSSIONS

Left ventricular outflow tract obstruction is a well-recognized complication following solely replacement of the mitral valve in HOCM. Contributing factors include the profile of the prosthetic valve, a reduced aorto -mitral angle, left ventricular hypertrophy, small ventricular cavity and thickening of the interventricular septum, all of each can result in a narrowed LVOT. In patients with a native mitral valve, such hypertrophy may remain subclinical and asymptomatic; however the presence of a prosthetic valve may exacerbate flow disturbance and lead to symptomatic obstruction. In this case, intraoperative inspection revealed a fibrous banding encircling the LVOT, together with severe interventricular septal hypertrophy, contributing further to flow restriction. We hypothesize that this band likely developed as a result of turbulent flow generated by prosthetic valve protrusion into the outflow tract. The prosthesis valve itself protruded into the LVOT, further compromising flow. This was clearly visualized in transthoracic echocardiographic images (oblique parasternal long-axis views, which demonstrated a thickened interventricular septum and the protruding mitral prosthesis.

Following the surgical procedure, our patient demonstrated excellent clinical recovery, with improved functional capacity and no recurrence of obstruction during follow-up.

CONCLUSIONS

Left ventricular myectomy remains the procedure of choice for patients diagnosed with hypertrophic obstructive cardiomyopathy.

Concomitant mitral valve repair and septal myectomy in patients with HOCM has been associated with favorable results, scientific data suggesting unfavorable outcome in patients who undergo only mitral valve replacement.

Surgical intervention in patients with severe obstructive hypertrophic cardiomyopathy is recommended to be performed in dedicated cardiovascular surgery centers with substantial experience and high operator volumes for this genetic pathology.

REFERENCES

1. *2023 ESC guidelines for the management of cardiomyopathies*
2. *Jones A, A Novel Case of Late Left Ventricular Outflow Tract Obstruction Post Mitral Valve Surgery 2019*
3. *Maigrot J.L, A Surgeon Toolkit for Mitral Valve Induced LVOT Obstruction with Minimal Septal Hypertrophy, 2025.*
4. *Nithiyanandhan P, 3DTEE Detection of Left Ventricular Outflow Tract Obstruction by Residual Native Mitral Leaflet following Mitral Valve Replacement in a Hypertrophic Obstructive Cardiomyopathy Patient , 2023*
5. *Zghal F. M. LVOT Obstruction after Mechanical Mitral Valve Replacement Research Trends and Challenges 2020*

THE COMMUNICATIONAL DIMENSION OF THE NURSE'S ROLE IN OPTIMIZING THE DOCTOR–PATIENT RELATIONSHIP: FROM HYPOTHESIS TO SCIENTIFIC EVIDENCE

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DOI: https://doi.org/10.66793/titum19proceeding3

Abstract

The study investigates the communicational dimension of the nurse's role in optimizing the doctor–patient relationship, based on the hypothesis that effective communication directly enhances the quality of medical care. A structured questionnaire was applied to a national sample of 560 nurses from different healthcare institutions. The data were analyzed in relation to demographic variables, professional experience, and perceptions regarding interdisciplinary collaboration. The findings highlight the central role of nurses in facilitating empathic and assertive communication, promoting mutual respect, and improving patient satisfaction. The results confirm that communication competence is not only a professional skill but also a determinant of therapeutic success. The paper concludes with recommendations for integrating communication training into continuous education and professional development programs for nurses, aiming to strengthen the human dimension of medical care.

Keywords: nurse, doctor–patient relationship, communication, professional competence, questionnaire

INTRODUCTION

Effective communication within the healthcare system is one of the key determinants of patient safety, satisfaction, and therapeutic success [1], [2]. The nurse, as an integral member of the medical team, represents the primary interface between the patient and the physician, ensuring continuity of care, accurate information transfer, and emotional support [3]. The communicational competence of nurses has become increasingly significant in the context of complex, technology-driven medical environments, where empathy, clarity, and relational adaptability are essential [4]. The professional literature emphasizes that the quality of the doctor–nurse–patient relationship directly influences adherence to treatment and the overall quality of care [5], [6]. Based on these premises, the present research investigates the communicational dimension of the nurse's role in optimizing the doctor–patient relationship, highlighting the professional, relational, and emotional factors that shape this interaction.

METHODOLOGY

The research was conducted between 3 August and 17 August 2025, as part of a study on professional and communicational competences of nurses in Romania. The main objective was to identify how nurses perceive their role in the doctor–patient communication process and to evaluate the interpersonal and professional factors influencing this perception. A quantitative, cross-sectional, descriptive, and comparative study was carried out based on a structured questionnaire distributed online and in printed format. The study sample consisted of 560 nurses from various healthcare institutions and specialties across Romania. Participation was voluntary and anonymous.

The questionnaire included 44 items structured into demographic and professional data, perceptions of professional

communication, collaboration with physicians, and training needs. Items were measured using a five-point Likert scale (1 = strongly disagree; 5 = strongly agree). The independent variables were gender, age, education, professional status, years of experience, specialty, and workplace type. The dependent variables referred to perceptions of communication roles, satisfaction with collaboration, and self-assessment of communication competence. Data were analyzed using descriptive statistics and comparative analysis across key variables.

RESULTS AND DISCUSSION

The study involved 560 nurses from different specialties. Most respondents were female (92.1%), confirming the gendered structure of the nursing profession (see Figure 1).

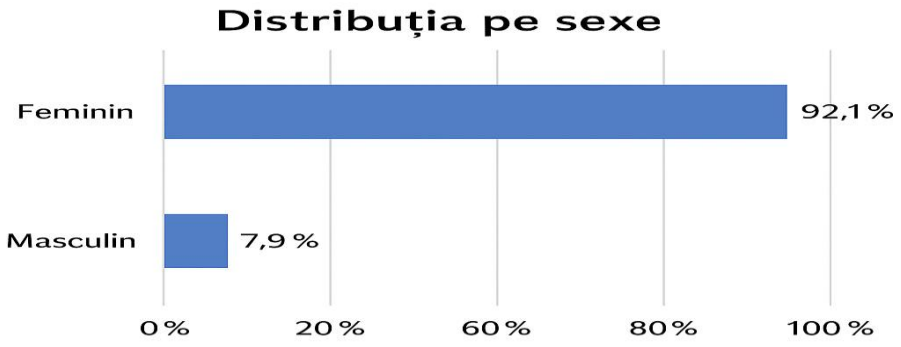

Figura 1. Distribuția respondenților după sex (n = 560)
Sursa: Chestionar 2025, elaborat de autori.

Figure 1. Distribution of respondents by gender (n = 560) Source: 2025 Nurses Questionnaire, authors' data.

Regarding educational background, 70% had post-secondary nursing school, 18% held a bachelor's degree, 10% a master's, and 0.5% a PhD (see Figure 2).

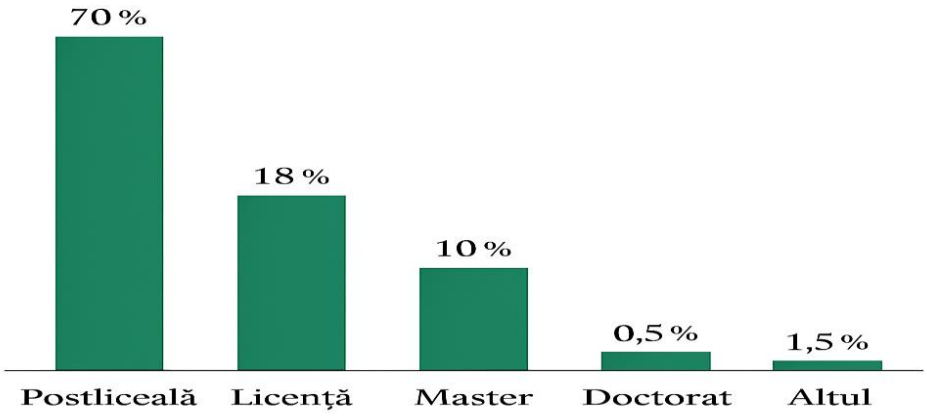

Figura 2. Nivelul de pregătire al asistenților medicali ($n = 560$)
Sursa: Chestionar 2025, elaborat de autori

Figure 2. Education level of nurses (n = 560) Source: 2025 Nurses Questionnaire, authors' data.

Professional experience was distributed as follows: 42.3% had more than 20 years, 25.2% between 4–10 years, 22.5% between 11–20 years, 6.6% between 1–3 years, and 2.9% less than one year (see Figure 3).

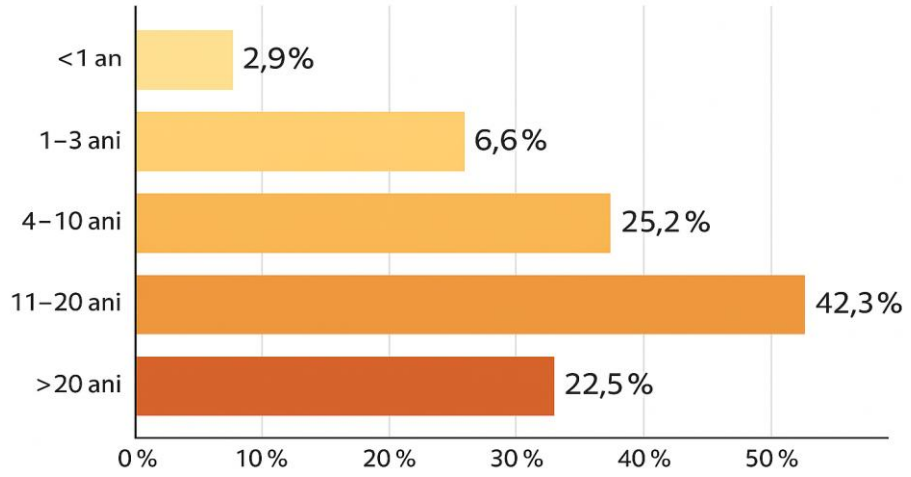

Figura 3. Distribuția respondenților după experiența profesională ($n = 560$)

Sursa: Chestionar 2025, elaborat de autori.

Figure 3. Professional experience distribution (n = 560) Source: 2025 Nurses Questionnaire, authors' data.

Most respondents considered themselves active in communication with patients, with 74% agreeing or strongly agreeing that they often act as intermediaries between doctors and patients (see Figure 4).

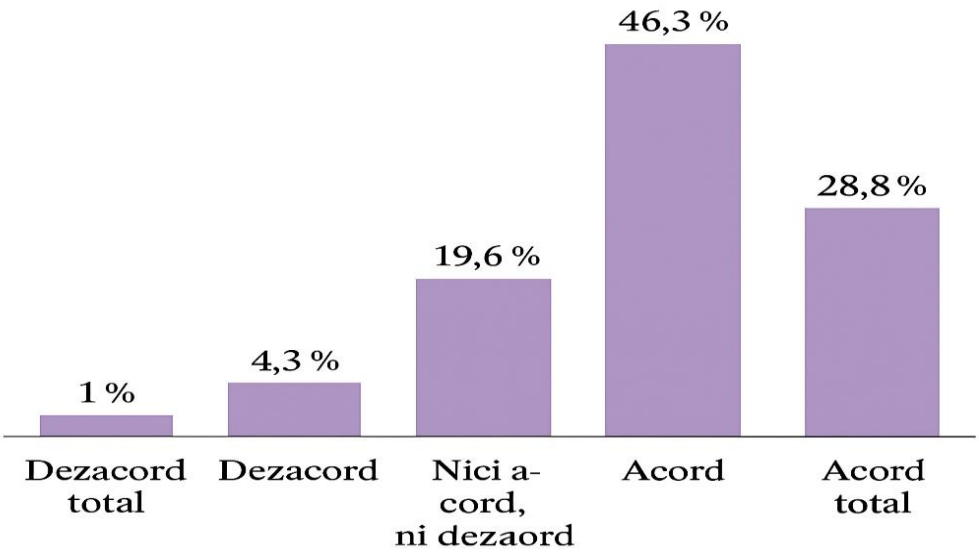

Figura 4. Rolul activ în comunicare ($n = 560$)

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Figure 4. Nurses' perception of their active role in communication (n = 560) Source: 2025 Nurses Questionnaire, authors' data.

Respondents' expectations from doctors focused primarily on respect and trust (70.9%), collaboration and communication (67.3%), and professional recognition (54.5%) (see Figure 5).

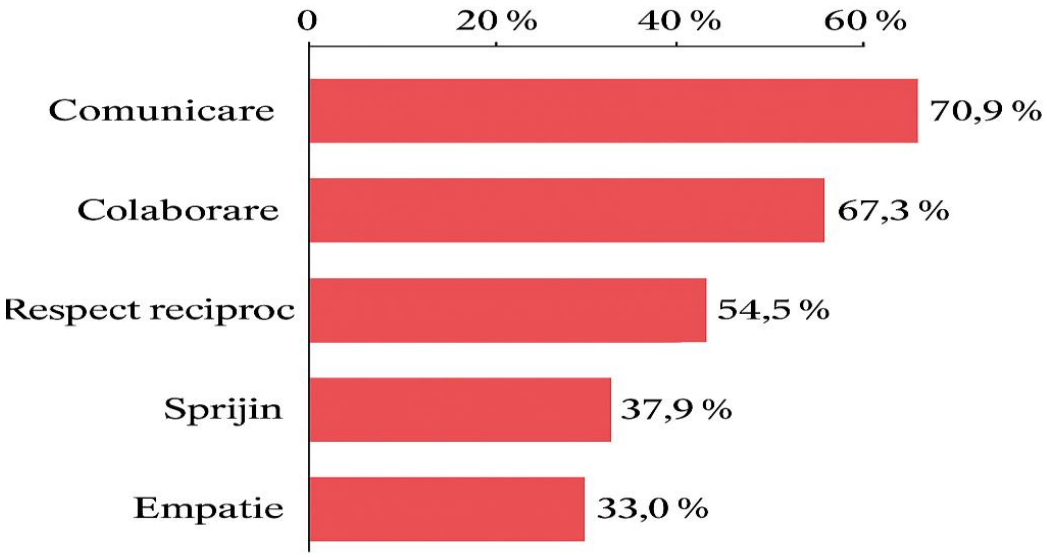

Figura 5. Aşteptările asistenţilor medicalí în raport cu medi

Sursa: Chestionar 2025, elaborat de autori

Figure 5. Nurses' expectations from doctors – main elements of collaboration (n = 560) Source: 2025 Nurses Questionnaire, authors' data.

Beyond the demographic variables already presented, the questionnaire also included several items related to age, professional status, workplace, and job satisfaction.

The age distribution showed that most respondents belonged to the 35–44 age group (36%), followed by 45–54 years (29%), confirming the professional maturity of the sample and the predominance of mid-career nurses in the healthcare system. Only 7% were under 25 years of age, which highlights the decreasing interest of young people in entering the profession.

Regarding professional status, 61% of the participants identified as general nurses, 26% as principal nurses, while the remainder were debutants or specialized nurses in critical care, pediatrics, or surgery. The data suggest a stable professional base with progressive specialization across the years of practice.

In terms of specialty and workplace, the majority (58%) worked in hospitals, 23% in primary care or family medicine, and 19% in private clinics or other medical facilities. The urban environment was dominant (76%), with 24% of respondents from rural areas. Nurses in rural settings reported higher workload and fewer opportunities for continuing education, but greater autonomy and closer patient contact.

An important section of the questionnaire referred to professional satisfaction and perceived appreciation.

While 68% of nurses described their job as "professionally fulfilling," nearly 45% stated that their efforts were not sufficiently acknowledged by management or medical leadership.

A smaller proportion (12%) reported experiences of conflict or communication breakdown with physicians or colleagues, often linked to hierarchical barriers or lack of feedback mechanisms.

These results, taken together, reinforce the idea that communication competence is interconnected with professional satisfaction, recognition, and institutional culture.

The findings support previous research showing that effective collaboration, clear role definition, and organizational respect significantly improve both teamwork and patient outcomes [5], [6], [7].

Qualitative responses confirmed these results, highlighting the importance of empathy, mutual respect, and communication in reducing patient anxiety and improving cooperation. Several nurses emphasized the need for improved interprofessional respect and listening to nurses' observations.

Only 32% had attended communication workshops, while over 90% expressed interest in future training, prioritizing empathy, conflict management, and communication with anxious or terminally ill patients. These findings underline the awareness of communicational competence and the lack of structured programs [2], [4].

CONCLUSIONS, CHALLENGES AND LIMITATIONS

The research confirmed that communication represents a fundamental pillar of nursing practice, directly influencing the efficiency of the doctor–patient relationship and the overall quality of care. Nurses perceive themselves as active communicators mediating understanding, reassurance, and compliance. The need to integrate structured communication training into both undergraduate and continuous professional education is evident. Challenges include the recognition of nurses' communicational role, workload constraints, and hierarchical barriers. Limitations concern self-reported data, regional concentration, and the lack of psychometric validation. Despite these, the study provides valuable empirical evidence on how nurses in Romania perceive and enact their communicational role, serving as a foundation for future research. The study provides an updated perspective on the communicational dimension of nursing practice in Romania, underlining the need for institutional strategies that foster collaborative communication across professional hierarchies. The findings may serve as a foundation for developing national guidelines and educational programs focused on communication in healthcare, and for future comparative studies at the European level.

REFERENCES

1. Peplau HE. Interpersonal Relations in Nursing. New York: Springer Publishing; 1991.

2. World Health Organization. Framework for Health Workforce Communication and Collaboration. Geneva: WHO Press; 2021.

3. Benner P. From Novice to Expert: Excellence and Power in Clinical Nursing Practice. Upper Saddle River, NJ: Prentice Hall; 2001.

4. Orem DE. Nursing: Concepts of Practice. 6th ed. St. Louis: Mosby; 2004.

5. Manojlovich M, DeCicco B. Healthy work environments, nurse–physician communication, and patients' outcomes. Am J Crit Care. 2007;16(6):536–543.

6. Leino-Kilpi H, Gröndahl W, Kaila M. Nurse–patient interaction and satisfaction. J Clin Nurs. 2019;28(1–2):230–241.

7. Ranjbar E, Kakemam E. Communication competence and its relation to quality of care in nurses. BMC Nurs. 2020;19(1):56.

8. World Health Organization. Patient Engagement for Safety and Quality of Care. Copenhagen: WHO Regional Office for Europe; 2017.

APPLICABILITY OF OZONE THERAPY IN RHEUMATOLOGY

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DOI: https://doi.org/10.66793/titum19proceeding4

Abstract: Rheumatology faces significant challenges in managing chronic pain and disability associated with musculoskeletal and autoimmune disorders, such as osteoarthritis and rheumatoid arthritis. since conventional treatments often carry systemic side effects and variable long-term efficacy, there has been a growing interest in minimally invasive options, among which medical ozone therapy has emerged. medical ozone, a mixture of oxygen and ozone, exerts its therapeutic effect through a controlled oxidation that activates the nuclear factor erythroid 2-related factor 2 signaling pathway. This action promotes redox homeostasis and produces a potent anti-inflammatory effect by suppressing pro-inflammatory cytokines (interleukin-1 beta and tumor necrosis factor alpha) while enhancing local tissue oxygenation. Furthermore, ozone stimulates tissue regeneration by upregulating growth factors (transforming growth factor beta, vascular endothelial growth factor). Clinical data strongly support the high efficacy of medical ozone therapy and a superior safety profile compared to invasive procedures. The treatment is administered both locally (intra-articular infiltrations) and systemically (autohemotherapy), often serving as an effective adjuvant to standard pharmacological agents. So, medical ozone therapy is a low-risk, conservative intervention that offers a substantial reduction in pain, a measurable improvement in physical function, and an overall increase in the quality of life for affected patients.

INTRODUCTION

Rheumatology, as a medical specialty focused on the diagnosis and treatment of musculoskeletal and autoimmune disorders, is constantly challenged by the need to manage chronic disease progression, persistent pain, and associated functional disability. Conventional treatments—primarily relying on non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and, ultimately, surgical interventions—are often associated with systemic side effects and variable long-term success rates. Within this complex therapeutic landscape, there has been a growing interest in minimally invasive and conservative approaches, among which medical ozone therapy (MOT) has emerged .

Medical ozone (O_3), a gas mixture composed of oxygen (95%) and ozone (5%), has established itself as a therapeutic modality with increasingly broad applicability in rheumatology, owing to its unique biochemical mechanisms. Unlike conventional pharmacological agents, ozone's action extends beyond simple anti-inflammation. Its primary effect is mediated by a controlled and transient oxidation, which induces an adaptive stimulation of the endogenous antioxidant system, primarily through the activation of the Nuclear factor erythroid 2-related factor 2 (Nrf2). This stimulation leads to an efficient modulation of the inflammatory response, significantly reducing the levels of key pro-inflammatory cytokines, such as Interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α), while simultaneously improving local microcirculation and tissue oxygenation (Viegas et al., 2019).

Consequently, clinical studies have consistently demonstrated that MOT, administered locally via intra-articular, periarticular, or intradiscal infiltrations, yields significant clinical benefits. These advantages translate into a marked reduction in pain - Visual Analogue Scale (VAS) scores and improvement in function - Oswestry Disability Index (ODI) and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scores, delivering outcomes comparable to more invasive procedures but with a superior safety profile and a negligible risk of complications (Zamboni et al., 2019). Thus, the applicability of MOT in rheumatology targets not only specific degenerative spinal conditions (disc herniation) but also a wide array of arthropathies and musculoskeletal pain syndromes. This versatility positions MOT as a promising first-line or adjuvant intervention within an integrated and multidisciplinary therapeutic management strategy.

1. CLASSIFICATION AND CHARACTERISTICS OF RHEUMATIC DISEASES IN THE ELDERLY

The medical approach to rheumatic diseases in the elderly population necessitates a distinct classification to account for age-related changes in immune function (immunosenescence) and the common presence of multiple co-existing conditions. This classification broadly delineates these disorders into two main groups: primary rheumatic pathologies in the elderly and pathologies with a late onset that present with an often

atypical or varied clinical picture. The first group includes diseases highly prevalent or unique to older age, such as Polymyalgia Rheumatica (PMR), characterized by symmetrical aching and stiffness, and the potentially sight-threatening Giant Cell Arteritis (GCA), which is a large-vessel vasculitis. Also central to this category is Osteoarthritis (OA), the most common joint disorder causing functional decline, as well as Osteoporosis, the leading metabolic bone disease responsible for fragility fractures. The elderly are also prone to certain crystal arthritides and paraneoplastic syndromes, where rheumatological symptoms may be the first sign of an underlying malignancy. The second group comprises established systemic autoimmune disorders presenting for the first time later in life, often known as Late-Onset Disease (LOD). These pathologies—including Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE), Spondyloarthropathies, Systemic Vasculitis, and Gout—often display a muted or altered symptomatic profile compared to their presentation in younger individuals, presenting significant diagnostic challenges and demanding a high index of clinical suspicion for timely management.

2. OZONE ACTION

MOT potentiates the positive effects of allopathic treatment and decreases its side effects. MOT achieves an endogenous cytokine therapy by stimulating the production of interferon beta (IFN-β). Additionally, ozone activates superoxide dismutase (SOD), which in turn neutralizes free oxygen radicals involved in mediating inflammation and leading to cartilaginous destruction. The stimulation of tissue regeneration is provided by the increased production of transforming growth factor beta (TGF-β). This is a transforming factor that will be released by macrophages and platelets precisely where tissue destruction processes are occurring (Lombardi et al., 2020).

MOT is administered both locally and systemically. Local administration involves subcutaneous infiltrations near painful joints, as well as intra-articular injections. Systemic administration is performed through major autohemotherapy, typically between specific flare-ups of rheumatoid arthritis. The standard course involves 15 sessions, twice a week, followed by maintenance sessions, depending on the patient's evolution.

At the molecular level, the therapy activates Nrf2, promoting redox homeostasis and reducing oxidative stress. Ozonolysis involves the interaction of ozone with the glutathione system, initiating a cascade of redox-based reactions that modulate intracellular signal transduction pathways and enhance cellular antioxidant regulation. By inducing ozone peroxidase activity and reinforcing the antioxidant defense systems, MOT decreases the production of proinflammatory cytokines and suppresses Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling activity (Galdiero et al., 2020).

Through these complex mechanisms, ozone modulates macrophage polarization and enhances antimicrobial efficiency. Ozone exposure activates macrophages and regulates the expression of inflammatory cytokines, thereby influencing both innate and adaptive immune responses. This process also promotes increased prostaglandin synthesis and bradykinin release, which collectively contribute to vasodilation, the modulation of inflammation, and pain control. Additionally, ozone improves oxygen metabolism by increasing oxygen delivery through erythrocytes and enhancing overall tissue oxygenation (De Faria et al., 2018).

Furthermore, MOT stimulates bone regeneration by upregulating the expression of critical growth factors such as vascular endothelial growth factor (VEGF) and transforming TGF-β, thus facilitating tissue repair and remodeling (Bosi et al., 2021). Collectively, these mechanisms contribute to accelerated tissue healing and the restoration of physiological function.

3. RHEUMATIC CONDITIONS THAT BENEFIT FROM OXYGEN THERAPY

3.1. Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic, progressive, systemic, and evolutive autoimmune inflammatory pathology of unknown etiology. It is primarily characterized by a symmetrical polyarthritis affecting the small joints of the hands and feet. If left untreated, the disease inevitably leads to cumulative joint destruction, resulting in substantial functional disability and a decrease in the patient's quality of life. The evolution of RA can be complicated by the functional impairment of articular, extra-articular, or systemic structures, which vary in severity. The prevalence of RA is estimated to range between 0.6% and 1.3% in the general population, with functional disability impacting up to 40% of patients, while the incidence of infectious complications in rheumatological pathologies is considered to be between 1.3-10%.

The underlying mechanism involves complex pathological interactions within the synovial membrane. Activation of T cells and macrophages plays a central role, driving inflammation and tissue destruction. Specifically, activated immune cells release cytokines such as Interleukin 17 (IL-17) and Interleukin 6 (IL-6), which, along with Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) signaling, stimulate Fibroblast-like Synoviocytes (FLS), which, in turn, facilitate bone erosion and cartilage destruction by activating osteoclasts and contributing to the formation of the destructive pannus (Miyazawa et al., 2017). Inflammation

also induces increased vascular permeability and facilitates the sustained migration of immune cells into the joint space.

The allopathic drug treatment that patients with rheumatoid arthritis follow consists of NSAIDs and analgesics, corticosteroids, and immunosuppressants, including drugs like methotrexate (MTX), leflunomide, sulfasalazine, hydroxychloroquine, or biological therapies such as infliximab, etanercept, or anakinra. In most cases, these patients have already undergone intra-articular corticosteroid injections, synovectomy, physiotherapy, kinesiotherapy, orthotics, or balneotherapy. However, the results have not been consistent, and most often, the side effects lead them to abandon the allopathic therapy.

A study that reviewed various pharmacological trials involving ozone in model diseases and clinical responses in RA patients (León Fernández et al., 2025) found that both methotrexate (MTX) and medical ozone share common mechanisms through adenosinergic regulation. The study proposed a novel pharmacological mechanism for treating RA, highlighting that the combination of MTX and medical ozone therapy reduces reactive oxygen species (ROS) overproduction, limits the generation of pro-inflammatory cytokines, and lowers anti-cyclic citrullinate peptide levels via a shared mechanism involving adenosine A1 receptors.

3.2. Ankylosing Spondylitis

Ankylosing Spondylitis (AS) is a chronic, systemic inflammatory disease primarily affecting the axial skeleton. It is characterized by inflammation of the sacroiliac joints and spine, enthesitis, and eventual structural damage and fusion of vertebrae in advanced cases. The pathogenesis of AS involves immune-mediated mechanisms, including elevated levels of pro-inflammatory cytokines (for example IL-17, TNF-α) and systemic activation of inflammatory cascades (Derrick et al., 2020). Clinically, patients present with persistent low back pain, stiffness—particularly in the morning—limited spinal mobility, reduced chest expansion and decreased quality of life.

O_3 complements conventional immunomodulatory and anti-inflammatory treatments in AS. A particularly relevant study (İnci & İnci, 2022) investigated the effect of MOT on systemic inflammatory biomarkers and clinical disease activity in 53 patients with AS (diagnosed according to the modified New York criteria) who had not changed their medical therapy for at least six months.

The intervention comprised MOT sessions and compared pre- and post-treatment values of neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), monocyte/lymphocyte ratio (MLR), C-reactive protein (CRP), the VAS for pain, the Bath Ankylosing Spondylitis Functional Index (BASFI) and the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). The results showed a statistically significant reduction in NLR, PLR, MLR, CRP and the clinical indices (VAS, BASFI, BASDAI) after MOT.

The authors additionally noted a positive correlation between changes in the hematologic biomarker ratios and disease activity, suggesting that MOT may modulate systemic inflammation in AS.

MOT is thought to exert anti-inflammatory, immunomodulatory and microcirculatory enhancing effects. In the context of AS, by reducing systemic inflammatory biomarker levels (NLR, PLR) and conventional cytokine-driven inflammation (reflected in CRP and VAS/BASDAI scores), ozone may help decrease enthesitic and axial inflammation, reduce pain and improve function. The study's findings provide preliminary empirical support to the hypothesis that MOT could serve as a beneficial adjunct in AS management, particularly when conventional therapy alone achieves sub-optimal control of pain, stiffness and functional limitation.

Although the study is promising, it remains relatively small and self-controlled rather than a large randomized placebo-controlled trial. Accordingly, clinicians and researchers must interpret results with caution and consider MOT as complementary, not a replacement for established Disease-Modifying Anti-Rheumatic Drugs (DMARDs) or biologic treatments in AS. Nonetheless, given the observed reductions in inflammatory indices and improvements in clinical outcomes, MOT holds potential as part of a multidisciplinary treatment strategy—offering patients an additional option to reduce pain, improve mobility and enhance quality of life in a condition where residual symptoms often persist despite standard therapy.

3.3. Fibromyalgia

Fibromyalgia syndrome (FMS) is a chronic musculoskeletal disorder characterized by widespread pain, fatigue, sleep disturbances, cognitive dysfunction, and heightened sensitivity to stimuli. Although the precise etiology remains unclear, FMS is widely regarded as a disorder of central pain processing, often accompanied by dysregulated inflammatory and oxidative pathways. Patients frequently report significant impairment in daily functioning and quality of life, and conventional pharmacologic therapies—such as analgesics, antidepressants, or neuromodulators—offer only partial relief, highlighting the need for complementary approaches.

In recent years, MOT has emerged as a promising adjunctive intervention for FMS. O_3 possesses anti-inflammatory, antioxidant, and modulatory effects on oxidative stress, which may help reduce chronic pain and improve energy metabolism at the cellular level. O_3 is administered through various methods, including major autohemotherapy, rectal insufflation, or localized injections, and is thought to induce controlled oxidative preconditioning that strengthens endogenous antioxidant defenses while attenuating systemic inflammation.

A relevant clinical investigation (Üşen A. et al., 2025) evaluated the short- and medium-term effects of major MOT on patients with fibromyalgia. This retrospective study included 83 patients diagnosed according to the American College of Rheumatology criteria and assessed changes in pain, fatigue, and functional capacity before and after a series of MOT sessions. The results demonstrated a statistically significant reduction in pain scores, as well as improvement in patient-reported fatigue and quality of life indices. Notably, improvements were observed within the first few treatment sessions and maintained at medium-term follow-up. The authors concluded that MOT may effectively complement conventional pharmacologic and rehabilitative interventions in FMS, particularly by modulating oxidative stress and systemic inflammation, which are central to the pathophysiology of the syndrome.

From a mechanistic perspective, MOT in fibromyalgia may act via several pathways. By enhancing tissue oxygenation, reducing excessive reactive oxygen species, and regulating inflammatory cytokines, ozone can contribute to decreased peripheral and central sensitization, which are believed to underlie the widespread pain and hyperalgesia characteristic of FMS. Furthermore, by improving microcirculation and metabolic efficiency, MOT may mitigate fatigue and enhance functional capacity.

Despite these promising findings, current evidence remains limited by small sample sizes, retrospective design, and short follow-up periods. Large, randomized controlled trials are needed to establish optimal dosing regimens, administration protocols, and long-term efficacy. Nonetheless, preliminary studies suggest that MOT is safe, well-tolerated, and potentially effective as part of a multimodal management strategy for fibromyalgia syndrome. As such, it represents a compelling complementary approach aimed at improving pain, fatigue, and quality of life in this challenging and often treatment-resistant condition.

3.4. Osteoarthritis

The prevalence of pain in adults aged 65 years or older varies by site, with low back pain, knee pain, and hip pain being the most common.

Osteoarthritis (OA) is a widespread musculoskeletal disease and a major cause of disability. More than 240 million people worldwide suffer from this condition. The pathogenesis of OA changes from a "wear and tear" theory to one involving chronic, progressive, low-grade inflammation, oxidative stress imbalance, and genetic involvement in the pathogenesis and progression of OA through compression-induced cell signaling, which regulates cartilage and subchondral bone homeostasis.

The prevalence of OA generally increases with age, being higher in the 65−74 age group (43,4%), and highest in the 75−84 age group (55%) (Kulkarni, 2014).

A randomized double-blind clinical trial (Arjmanddoust, Nazari & Moezy, 2025) investigated the efficacy of intra-articular MOT in patients with knee OA. Conducted at a university hospital in Tehran, Iran, the study sought to determine whether medical ozone injections could effectively reduce pain and improve joint function in individuals with moderate osteoarthritic degeneration. A total of 59 patients, aged between 50 and 75 years, diagnosed with primary knee OA (Kellgren–Lawrence grade II or III), were enrolled. Participants were randomly assigned to three groups: two treatment groups receiving intra-articular ozone at concentrations of 20 µg/mL and 40 µg/mL, respectively, and a control group receiving medical oxygen. Each participant underwent four weekly intra-articular injections, and follow-up assessments were performed at two weeks, one month, and two months after the final treatment session.

Clinical outcomes were measured using validated instruments, including the VAS for pain, the WOMAC for pain, stiffness, and function, the Timed Up and Go test (TUG), the Six-Minute Walk Test (6MWT), and measurements of knee range of motion.

The results demonstrated that both ozone-treated groups experienced a significant reduction in pain scores and improvement in functional mobility compared with the control group. Patients reported reduced stiffness, greater ease of movement, and overall improvement in quality of life. Interestingly, no statistically significant differences were observed between the two ozone concentrations, suggesting a potential threshold effect, whereby a moderate concentration (20 µg/mL) achieves optimal clinical benefit without requiring higher doses. The treatment was well tolerated, with no major adverse effects reported.

The authors concluded that intra-articular MOT represents a safe and effective therapeutic option for patients with moderate knee OA, particularly in earlier disease stages where functional improvement remains achievable. They emphasized, however, that the observed benefits were assessed over a relatively short follow-up period (two months), underscoring the need for larger, long-term trials to confirm both the durability of outcomes and the safety profile of repeated ozone administration.

Overall, this study adds meaningful evidence to the growing body of literature supporting MOT as a promising adjunctive intervention in musculoskeletal and degenerative joint diseases. By applying a rigorous randomized design and directly comparing two therapeutic concentrations, the research highlights ozone's potential role as a complementary and cost-effective alternative within modern rheumatologic practice.

3.5. Systemic Sclerosis

Systemic sclerosis (SSc) is a complex autoimmune connective-tissue disease characterised by widespread vascular damage, immune activation and progressive fibrosis of the skin and internal organs. Patients typically present with skin thickening, Raynaud's phenomenon, limited joint mobility, and in advanced cases digital ulcers, pulmonary arterial hypertension and interstitial lung disease. The pathophysiology involves vascular endothelial injury, persistent inflammation, oxidative stress and fibroblast activation leading to collagen over-deposition and tissue stiffening. Clinically, SSc is among the most challenging rheumatic conditions in terms of therapeutic management, functional impairment and quality of life.

MOT has been explored as potential complementary approaches in SSc. Ozone exerts a spectrum of effects including anti-inflammatory, antioxidant, improvement of microcirculation and modulation of immune responses. These mechanisms are particularly relevant in SSc where oxidative stress, endothelial dysfunction and tissue hypoxia play major roles. By enhancing local oxygenation, reducing reactive oxygen species (ROS), upregulating antioxidant systems and improving peripheral blood flow, MOT may help address key pathological processes in SSc—especially in the microvascular and fibrotic components of the disease (Turrisi et al., 2020).

A compelling study (Kaymaz S. et al., 2022) evaluated the efficacy of local oxygen–ozone therapy in patients with SSc who had refractory digital ulcers (DUs). In this randomised controlled trial, 25 SSc patients were allocated to the ozone group (n = 13) or control group (n = 12); the ozone group received standard medical treatment plus local O_2–O_3 therapy, while the control group received standard treatment alone. After four weeks of treatment, the ozone group demonstrated a 92% efficacy rate, significantly higher than the 42% observed in the control group (P = 0.010). Moreover, hand function assessed by the Health Assessment Questionnaire (HAQ) and the Modified Hand Mobility in Scleroderma (HAMISm) test improved significantly in the ozone-treated cohort. This study highlights ozone therapy's ability to enhance healing of difficult ulcers, improve hand mobility, and reduce functional disability in SSc.

From a practical perspective, the addition of MOT in SSc may provide meaningful functional benefits—particularly by improving peripheral vascular perfusion, reducing microvascular dysfunction and possibly slowing progression of sclerotic changes. For patients facing chronic complications such as digital ulcers and joint contractures, MOT offers a complementary tool that may ease symptoms, enhance mobility and improve quality of life. However, it is important to stress that while the initial evidence is promising, larger-scale controlled trials with longer follow-up are needed to define optimal dosing, safety, long-term efficacy and integration within standard rheumatologic care.

In summary, systemic sclerosis remains a severe rheumatic disease with limited curative options, but the incorporation of MOT—through modulation of oxidative stress, improvement of microcirculation and immune regulation—emerges as a promising adjunctive strategy. With growing clinical evidence such as the digital ulcer Randomized Controlled Trial (RCTs), MOT may become a valuable component of multidisciplinary management in SSc, helping patients reduce ulcer burden, improve hand function and withstand the vascular/fibrotic challenges of the disease.

CONCLUSIONS

MOT holds significant promise as an adjunctive treatment in the management of various rheumatological diseases, including RA, AS, OA, SSc, and fibromyalgia. The therapy's multifaceted mechanisms—ranging from the modulation of oxidative stress and reduction of pro-inflammatory cytokines to the enhancement of tissue oxygenation and microcirculation—suggest its potential in improving pain, functional mobility, and overall quality of life for patients suffering from chronic, degenerative musculoskeletal and autoimmune disorders.

A key advantage of MOT lies in its ability to significantly reduce inflammation and alleviate pain through natural physiological processes. Studies have shown that ozone therapy can positively influence inflammatory markers, contributing to a reduction in pain and an overall improvement in functional mobility. The therapy's capacity to promote vascular health and microcirculation is particularly beneficial for patients with osteoarthritis, as it can help restore blood flow to damaged tissues, supporting healing and regeneration. These mechanisms help patients recover functionality and reduce the impact of disease symptoms on their daily lives.

Moreover, MOT has demonstrated a notable ability to support tissue regeneration and enhance the healing process, particularly in chronic inflammatory conditions where healing is often compromised. This regenerative effect is especially important for diseases like osteoarthritis, where joint cartilage deterioration can lead to irreversible damage. By stimulating antioxidant defenses and reducing oxidative stress, ozone therapy aids in tissue repair, slows down degenerative processes, and may even improve the long-term structural integrity of affected joints.

One of the most promising aspects of MOT is its low-risk profile, with minimal side effects reported in clinical practice. This makes it an ideal option for elderly patients, those with comorbidities, or individuals who might be more sensitive to the side effects of conventional pharmacological treatments. In these populations, MOT provides a safer alternative with fewer systemic effects. Additionally, its versatility in addressing multiple

facets of disease, from pain relief to tissue repair, allows for a more holistic approach to chronic rheumatic conditions, improving both symptomatic relief and quality of life.

Furthermore, MOT's role in modulating immune function has gained increasing attention in the treatment of autoimmune diseases such as RA and SSc. By enhancing the body's natural antioxidant defenses and reducing the production of pro-inflammatory cytokines, MOT can help regulate the immune system, offering a potential therapeutic strategy to address the root causes of autoimmune disease activity. This immunomodulatory effect makes MOT a powerful tool in managing diseases where immune dysregulation plays a key role in the disease process.

In addition to its therapeutic effects, the cost-effectiveness and ease of application of MOT make it an appealing option for both clinicians and patients. Compared to more invasive or expensive treatments, ozone therapy is non-invasive and generally well-tolerated, with treatment sessions being relatively short. This can translate to a more efficient use of healthcare resources and better patient adherence to treatment plans, especially for those managing chronic conditions over an extended period.

Finally, MOT offers a promising, safe, and innovative approach to managing rheumatic diseases. The growing body of evidence supports its effectiveness in reducing inflammation, alleviating pain, improving functional mobility, and enhancing tissue regeneration. As clinical experience with MOT continues to expand, its potential to become a cornerstone of multidisciplinary treatment strategies for chronic musculoskeletal and autoimmune disorders is increasingly recognized. By incorporating MOT into treatment protocols, healthcare providers can offer patients a more comprehensive and individualized approach, addressing both the symptoms and underlying mechanisms of disease, ultimately improving patient outcomes and quality of life.

REFERENCES

1. *Arjmanddoust, Z., Nazari, A. & Moezy, A. (2025). Efficacy of two doses of intra-articular ozone therapy for pain and functional mobility in knee osteoarthritis: a double-blind randomized trial. Advances in Rheumatology, Mar 6;65(1):11.*
2. *Bosi, S., et al. (2021). Medical ozone in musculoskeletal regenerative medicine. Musculoskeletal Science and Practice, 52, 101459.*
3. *De Faria, M. M., et al. (2018). Ozone therapy in the treatment of pain and inflammation: Role of macrophages and cytokines. Free Radical Biology and Medicine, 125, 155-167.*
4. *Derrick, A., et al. (2020). Spondyloarthritis: Pathogenesis and clinical manifestations. Best Practice & Research Clinical Rheumatology, 34(5), 101547.*
5. *Galdiero, M., et al. (2020). Ozone therapy: A novel approach for the treatment of inflammation and oxidative stress. Biological Chemistry, 401(3), 281-296.*
6. *İnci, H. & İnci, F. (2022). Effect of ozone therapy on neutrophil/lymphocyte, platelet/lymphocyte ratios, and disease activity in ankylosing spondylitis: a self-controlled randomized study. Medical Gas Research, 13(2):53-58. doi:10.4103/2045-9912.344981.*
7. *Kaymaz, S., et al. (2022). Efficacy of local oxygen–ozone therapy for the treatment of digital ulcer refractory to medical therapy in systemic sclerosis: a randomized controlled study. Modern Rheumatology, 32(6):1102-1107. doi:10.1093/mr/roab117.*
8. *Kulkarni, A. (2014). Osteoarthritis prevalence, disease burden and economic impact. Best Practice & Research Clinical Rheumatology, 28(1), 1-10.*
9. *León Fernández, O. S., et al. (2025). Medical Ozone Increases Methotrexate Effects in Rheumatoid Arthritis Through a Shared New Mechanism Which Involves Adenosine. International Journal of Molecular Sciences, May 29;26(11):5256. doi: 10.3390/ijms26115256. PMID: 40508064; PMCID: PMC12155193.*
10. *Lombardi, C., et al. (2020). The use of medical ozone in the treatment of musculoskeletal disorders. Musculoskeletal Science and Practice, 47, 40-48.*
11. *Miyazawa, K., et al. (2017). Macrophages and T cell activation in rheumatoid arthritis. Clinical Immunology, 183, 31-39.*
12. *Turrisi, G., et al. (2020). Ozone therapy in systemic sclerosis: An innovative approach to modulate inflammation and fibrosis. International Journal of Rheumatic Diseases, 23(4), 478-486.*
13. *Üşen, A., et al. (2025). Short- and Medium-Term Effects of Major Ozone Therapy on Disease Parameters in Fibromyalgia Syndrome: A Retrospective Study. Rheumatology International, 45:72. doi:10.1007/s00296-025-05827-1.*
14. *Viegas, M. P., et al. (2019). The role of ozone in modulating inflammation and oxidative stress in chronic diseases. Free Radical Biology and Medicine, 134, 309-317.*
15. *Zamboni, P., et al. (2019). Medical ozone therapy in the management of osteoarthritis: A systematic review and meta-analysis. Osteoarthritis and Cartilage, 27(5), 670-679.*

ASSESSMENT TECHNIQUES AND SPECIFIC CARE AT PATIENTS WITH PERITONITIS

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DOI: https://doi.org/10.66793/titum19proceeding5

Abstract

Acute diffuse peritonitis is a major surgical emergency, which, in the absence of adequate treatment, evolves to death within 5-7 days in common forms or within 2-3 days in severe forms. The patient is urgently admitted to the intensive care unit of the general surgery department. The study group included 42 patients, of which 27 with acute peritonitis due to appendiceal perforation, 6 patients with acute peritonitis due to duodenal ulcer perforation and 9 patients with abscessed appendiceal plastron. Surgical treatment was associated with antibiotic treatment: pre-, intra- and postoperative, as well as with general supportive measures associated with specific appropriate nursing care, so that the evolution was favorable and with as few complications as possible. The interventions performed were: appendectomy with peritoneal lavage and multiple drainage in 27 cases; ulcer suture with epiploonoplasty, peritoneal lavage and multiple drainage in 6 cases; peritoneal cavity lavage and multiple drainage in 9 cases with abscessed appendicular plastron. The major objective of the study was to provide data on the contribution of nursing in the treatment of patients with acute peritonitis, as well as its role in the therapeutic scheme in order to achieve a favorable postoperative evolution and a superior quality of life.

Key words: peritonitis, sepsis, lavage, drainage, nursing interventions

Peritonitis is the inflammation of the peritoneal serosa, a membrane that covers the abdominal organs and the inner surface of the abdominal walls. The peritoneal space is a "virtual" space located between the visceral peritoneum (the one that covers the abdominal organs, constituting their serosa) and the parietal peritoneum (which lines the abdominal walls on the inside).

Normally, in the peritoneal space, there is a minimal amount of fluid, which allows the two peritoneal membranes to slide freely over each other. In pathological conditions, a larger amount of fluid accumulates in the peritoneal space or various fluids are accidentally discharged (bile, gastric juice, intestinal fluid, pus, etc.).

Depending on the extension of the inflammatory process, peritonitis is divided into localized peritonitis (the infection is limited to an area of the abdomen) and generalized peritonitis. Untreated, localized peritonitis leads to the formation of an abscess, and in the generalized form, microbes can reach the blood (septicemia), causing septic shock. If no intervention is made, the patient dies. This is how the particular seriousness of this disease is understood. The moment of surgery should not be postponed, because the prognosis is all the better, the more promptly the intervention is carried out. The condition is more severe in the elderly, with a more precarious defense of the body. In them, the diagnosis is more difficult, given the more blurred clinical picture.[3]

Surgical treatment must be framed by antibiotic treatment: pre-, intra- and postoperative, as well as general supportive measures. The intervention aims to evacuate the infected fluid from the peritoneal cavity, remove the source of contamination (if necessary, suture the perforation, remove the organ - appendix), lavage of the cavity with large amounts of antiseptic solution and multiple peritoneal drainage with drain tubes. The pathophysiological disorders in the evolution of acute diffuse peritonitis have been systematized in 3 phases or stages of peritoneal irritation, declared peritonitis and neglected peritonitis.

Peritenal irritation is the initial phase of diffuse acute peritonitis, which usually spreads during the first 6 hours after onset. It is considered reversible. In chemical peritonitis, as a result of irritation of the peritoneal interoreceptors by the intraperitoneal digestive effusion from a perforation of an irritating organ (gastroduodenal content, bile, pancreatic enzymes, colonic content), an abundant exudate rich in fibrin and antibodies appears, which tend to limit the inflammatory process. The brutal excitation of the peritoneal interoreceptors explains the intensity of the pain and the appearance of inflammation, simultaneously with the initiation of reflex mechanisms: contracture of the abdominal muscles and paralysis of the digestive muscles. The greater omentum, the true "gendarme" of the peritoneal cavity, is attracted by chemotaxis to the site of maximum irritation and adheres to the perforation. If the perforation is covered and completely sealed, the peritonitis remains in this phase and usually progresses to remission, a situation in which emergency surgery is no longer necessary. The classic example is that of a covered ulcerative perforation.[4] Manifest peritonitis or purulent peritonitis continues the peritoneal irritation phase. The presence and multiplication of pathogenic microbial agents in the peritoneal cavity profoundly disrupts the functions of the peritoneum through irritation of the peritoneal interoreceptors, progressive paralysis of the muscles of the entire digestive tract and alteration of intestinal digestion and absorption. Clinically manifest peritonitis is characterized by the appearance of purulent peritonitis with false membranes, concomitantly with the onset of hypovolemic and toxicoseptic shock.[3]

The paralysis of the muscles of the digestive tract, which occurred in the previous phase, paralytic dilatation of the intestinal loops produces a large amount of fluid and gases. The sequestration of water and electrolytes in the lumen and walls of the digestive tract and in the loops causes massive fluid retention in the peritoneal cavity (Randal space III), to which are added losses through sweating, vomiting, perspiration (hypovolemic shock), causing a decrease in circulating blood volume and severe hydroelectrolytic imbalances, with the appearance of extracellular dehydration associated with intracellular dehydration.

The modification of hydrostatic pressure and colloidosmotic pressure (protein loss in space III) profoundly affects local and systemic transcapillary exchanges. Electrolyte disorders are secondary to the loss of sodium, potassium, chlorine and magnesium ions. Hyposodemia, hypokalemia, hypochloremia (predominant loss through vomiting and sweating) and hypomagnesemia (responsible for nervous phenomena) are established. Cell membrane exchanges are affected, with the replacement of extracellular Na+ with intracellular K+ (ATPase-K+ dependent Na+ and K+ pump) and the development of metabolic acidosis. Non-protein nitrogen increases (hypovolemia but renal hypoirrigation, hypercatabolism) and hematocrit due to dehydration. [4] Transcapillary and transmembrane exchange disorders are exacerbated and aggravated by the over-addition of systemic toxemia (through the resorption of microbial exotoxins) which will initiate the cascade of the systemic inflammatory response, with the appearance of toxicoseptic shock.

Neglected peritonitis is the advanced evolutionary phase of diffuse peritonitis, which follows untreated declared peritonitis and usually appears after 36-48 hours from onset. It is marked by the installation of paralytic occlusion and on a systemic level, with the appearance of multiple organ failure (MOF). Practically all organs are affected to a variable degree and in various associations, but in the final stage irreversible multi-organ failure sets in. The most frequently encountered components of MOF are acute respiratory failure, cardio-respiratory failure, and acute renal failure.[3]

Respiratory failure is due to both diaphragmatic immobility through phrenic contracture (irritation of the diaphragmatic peritoneum) and abdominal distension (paralytic intestinal occlusion), as well as hydroelectrolytic disorders.

Cardio-circulatory failure is the consequence of hypovolemia, which causes hemodynamic changes, with a progressive reduction in venous return to the right heart, until the appearance of cardiorespiratory arrest. It is caused, at first, by tissue hypoperfusion.

Subsequently, tissue ischemia characteristic of shock sets in. Massive fluid losses with hemoconcentration and stasis in the microcirculation sector favor the appearance of microthromboses, an aggravating factor by

accentuating peripheral hypoxia. In addition, the opening of arteriovenous shunts is added, which further reduces the amount of blood reaching the tissues.

Rheological disorders in the microcirculation culminate in the appearance of disseminated intravascular coagulation (DIC syndrome).

Acute renal failure is the result of renal hypoperfusion and is manifested by the appearance of oliguria and then anuria, concomitantly with an increase in serum urea and creatinine. Bacterial toxins cause damage to the nephrons, with the possible appearance of organic renal failure.

Adrenal insufficiency is also the consequence of hypoperfusion of the adrenal medulla and adrenal cortex; thus, a massive discharge of adrenaline and noradrenaline into the circulation occurs, which causes strong vasoconstriction, with increased hypoxia and exacerbation of metabolic tissue changes through a "vicious circle" mechanism. The intensity and grouping of clinical manifestations may suggest the phase of evolution of the peritonitis process. In the peritoneal irritation phase, the patient presents with pain and abdominal contracture. The pain is more intense at the level of the lesion and may radiate to the shoulder, interscapulo-vertebral or dorsal region. In acute gastroduodenal perforations it may become shock-like, due to the brutal irritation of the peritoneal interoceptors (vagal shock). The abdominal contracture may be less marked at the onset, but becomes pronounced within a few hours. The patient also presents with reflex vomiting, initially alimentary, which may then be bilious. Thirst may occur as a result of fluid loss, hiccups and, after a few hours, paralytic ileus. The patient presents with positive Blumberg, Dieulafoy, Mandel and Grassman signs. He may also present with oliguria, fever, leukocytosis (over 12000-15000/mm3), with neutrophilia. The phase of declared peritonitis (peritoneal shock phase) usually sets in after 6 hours from the onset. In this phase, the pain decreases in intensity and meteorism, poraceous vomiting appear (due to paralytic ileus). The signs of Blumberg, Dieulafoy and Mandel decrease in intensity, and the Grassman sign is more intense.[2]

The muscular contraction gradually attenuates being replaced by the progressive abdominal distension of the paralytic ileus. Intestinal transit is completely suppressed, both for feces and for gases. Important hydroelectrolytic disorders are established with a decrease in serum Na+, K+, Mg2+ and Cl-. Hemoconcentration occurs, with an increase in hematocrit, metabolic acidosis and extrarenal azotemia. The patient presents with fever and hyperleukocytosis. In the neglected peritonitis phase, the patient presents a Hippocratic facies and is restless, agitated, but conscious. The abdomen is relaxed, without muscle contracture and without significant pain. The biological state is profoundly affected by multiple organ insufficiency. The patient presents with fecal vomiting (a sign of severe prognosis), scleral subicterus due to liver insufficiency, anuria, tachypnea, cyanosis (due to bloating and diaphragm immobility), dry tongue (a sign of intense dehydration).

To evaluate the prognosis, the APACHE II severity prediction score is calculated, based on the comparative evaluation of biochemical parameters determined upon admission and during evolution, and the Glasgow score.[1] Paraclinical explorations relevant in peritonitis are radiological and ultrasound.

Plain abdominal radiography is extremely useful in the diagnosis of perforations of the cavitary viscera. In peritonitis due to gastroduodenal or colic perforations, pneumoperitoneum is evident, which, in orthostatism, appears as a thin air layer that appears between the liver and diaphragm, and if it is more abundant, also between the spleen and diaphragm.

Abdominal ultrasound is indicated for differential diagnosis; in peritonitis, it can reveal the presence of exudate in the abdominal cavity and/or immobility of intestinal loops. CT and MRI reveal the existence of an abdominal pathology.[5]

Acute diffuse peritonitis is a major surgical emergency, which, in the absence of treatment, evolves to death in 5-7 days (common forms) or in 2-3 days in severe forms. The patient is urgently admitted to the intensive care unit of the surgery department. Treatment must be early (from the onset) and complex, aiming at the following

therapeutic objectives: suppressing the source of peritoneal contamination, combating infection and toxicoseptic resorption through antibiotic therapy, toileting and draining the peritoneal cavity, correcting volemic, hydroelectrolytic and acid-base disorders through perioperative intensive therapy, preventing or reducing possible complications. The major therapeutic objectives (elimination of the source of contamination and peritoneal drainage) are met by surgical treatment, which is indicated urgently and as early as possible from the onset of diffuse peritonitis. In the postoperative period, sustained treatment of the patient is imperative with the use of vasopressors and cardiac stimulants as needed, in parallel with a sufficient fluid intake to maintain diuresis at a level of at least 2000 ml/ 24 hours and with the resumption of intestinal transit as soon as possible postoperatively.[5]

Severe sepsis cannot always be controlled only through a single intervention, requiring continuous peritoneal lavage and in certain situations scheduled interventions to prevent the onset of multiple organ failure syndrome (MSOD – MSOF). The study group consists of patients with peritonitis hospitalized between January 2022 and January 2025 in the Surgery Department of Turceni Hospital.

The study is retrospective and was conducted based on the analysis of observation sheets, diagnostic and treatment protocols, admission or transfer registers, but also on clinical observation during internships carried out in this department.

Patients who presented to the emergency room with peritonitis and life-threatening cardiovascular pathology or with advanced hepato-renal pathology who were transferred to hospitals with a higher level of competence and where there was the possibility of permanent pre- and postoperative monitoring in intensive care units and the possibility of performing appropriate treatment in order to prevent multiple organ failure syndrome were not included in the clinical study.

The study group included 42 patients, of whom 27 (64.28%) had acute peritonitis due to appendiceal perforation, 6 (14.28%) had acute peritonitis due to duodenal ulcer perforation and 9 patients (21.42%) had abscessed appendiceal plastron. 29 patients were male (69.04%) and 13 patients were female (30.95%). The major objective of the study was to provide data on the contribution of surgical nursing in the treatment of patients with acute peritonitis, as well as its role in the therapeutic scheme in order to achieve a favorable postoperative evolution and a superior quality of life.

The specific short-term objectives were: the patient to benefit from emergency care to favor the evolution and minimize the risk of complications; the patient to be volemic and hydroelectrolytic balanced; to have physiological eliminations; to have normal and efficient breathing and circulation; the patient to have physiological and restful sleep.

The specific medium-term objectives were: the patient to mobilize and adopt comfortable positions; to have a satisfactory evolution, without complications; the patient to acquire the knowledge necessary to regain health.

Specific long-term objectives: the patient to present a state of physical well-being; the patient to reintegrate socially as quickly as possible. The data source was: observation sheets of patients with Following the evaluation of the nursing process in the study group, a favorable evolution was found without occlusive phenomena in the postoperative period, without cardiovascular or metabolic decompensations and alterations in the postoperative biological status.

The objectives of the preoperative treatment were: restoration of volemia, antibiotic therapy with cephalosporins in association with Metronidazole, nasogastric suction tube, monitoring of vital functions and anti-pain medication.

The interventions performed were: appendectomy with peritoneal lavage and multiple drainage in 27 patients (64.28%); ulcer suture with epiploonoplasty, peritoneal lavage and multiple drainage in 6 patients (14.28%); lavage of the peritoneal cavity and multiple drainage in 9 patients (21.42%) with abscessed appendicular plastron.

The intra-abdominal access method was represented by median laparotomy in 31 cases (73.80%) and by Jalaguier-type incision in 11 cases (26.19%). The resumption of intestinal transit was achieved in the first 48 hours from the moment of surgery in 25 cases (50.52%) and in 17 cases (40.47%) at 72 hours postoperatively by stimulating transit with Myostin and evacuating enemas.

Postoperative complications were represented by the presence of parietal suppurations in 15 patients (35.71%) with a simple evolution that did not require the extension of the hospitalization period by more than 4 - 5 days under targeted antibiotic therapy according to the antibiograms performed. The hospitalization interval of the patients was between 8 and 10 days in 19 cases (45.23%) and between 6 and 7 days in 23 cases (54.76%). No postoperative eventrations or other postoperative incisional defects were recorded after the surgical interventions performed.peritonitis; diagnostic and treatment protocols; registers of admissions and interhospital transfers.

CONCLUSIONS

Acute peritonitis represents the inflammatory reaction of the peritoneal serosa, diffuse or localized, of infectious or not origin, produced by varied and complex mechanisms. The diagnosis in acute peritonitis is a complex diagnosis, carried out according to an algorithm, which involves the establishment in successive stages of a positive, differential and etiological diagnosis.
The positive diagnosis is based on the anamnesis, the objective examination and the paraclinical investigations within which the abdominal radiography on empty is extremely useful in the diagnosis of perforations of the cavitary viscera and the abdominal ultrasound is indicated for the differential diagnosis, by highlighting the exudate in the abdominal cavity and/or the immobility of the intestinal loops.
Treatment must be early from the onset and complex, aiming at the following therapeutic objectives: suppression of the source of peritoneal contamination, combating infection and toxicoseptic resorption through antibiotic therapy, toileting and drainage of the peritoneal cavity, correction of volemic, hydroelectrolytic and acid-base disorders through perioperative intensive therapy, prevention or reduction of possible complications.
The surgical intervention is performed urgently, with general anesthesia, after several hours of preoperative preparation, the duration of which depends on the interval from the onset and, especially, on the biological terrain, but without exceeding 6 hours.
Surgical treatment must be strictly individualized for each case, depending on the nature of the peritonitis, the time elapsed since the onset, the patient's general condition, age and the existence of concomitant diseases. It must be doubled by pre-, intra- and postoperative intensive therapy, which corrects the serious imbalances of peritonitis.
The evolution and prognosis of the patient with peritonitis are determined by early surgical treatment and sustained hemodynamic rebalancing.

REFERENCES

1. Funariu G.(2002), Abdominal Surgery, Dacia Publishing House, Cluj Napoca, p.386-393.

2. Ghelase F., editor, (2013), Surgery, vol. III. Surgical Pathology, Sitech Publishing House, Craiova, p. 109-113.

3. Popescu I., Vasilescu C., editor, (1998), Peritonitis. Celsius Publishing House, Bucharest, p. 14-76, 253-265.

4. Ungureanu F.D.(2016), General Surgery Course, Vol. I, 3rd Edition, Hamangiu Publishing House, Bucharest, p. 109-113.

5. Trașcă E. under editorship, (2013), Notions of General Nursing and in Surgical Specialties, Craiova University Medical Publishing House, p. 539-541.

THERAPEUTIC APPROACH TO PATIENTS WITH LOWER LIMB AMPUTATIONS

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DOI: https://doi.org/10.66793/titum19proceeding6

Abstract

The pain experienced by patients and the behaviors associated with this injury will then be presented, as well as psychological comorbidities such as mood disorders and adjustment difficulties. The work of mourning is long and emotionally demanding. You may experience all sorts of emotions. But know that there is no right or wrong reaction. Each person is unique and reacts in their own way. What you feel and express will gradually allow you to let go of who you were "before" and envision who you want to be "now." Amputation triggers numerous psychological processes in the person who undergoes it. Psychological support offered in specialized facilities complements rehabilitation, helping the individual to overcome shock and emotional distress, cope with trauma, and regain their place in daily life.

Introduction

As a preamble to this article, it is important to note from the outset that amputation and its consequences are rarely addressed in the literature. The objective of this article, however, is to provide an overview of the issues surrounding amputation, drawing on the existing literature and beginning with some epidemiological data. The pain experienced by patients and the behaviors associated with this injury will then be presented, as well as psychological comorbidities such as mood disorders and adjustment difficulties. Finally, we will discuss potential interventions and, in particular, the various aspects of psychological work with amputees. This chapter will be illustrated with excerpts from interviews with amputees and professionals working in the field of amputation.

Although upper limb amputation (hand, forearm, upper arm) is often more disabling than lower limb amputation (foot, leg, thigh, pelvis) for various reasons (balance problems in the case of arm amputation, possible loss of the dominant hand), this chapter will focus on lower limb amputation, as it accounts for 86% of amputations, compared to 14% for upper limb amputations. It should be noted, however, that most of the points discussed (apart from epidemiological data) are applicable to both types of amputation.

Epidemiological data concerning amputation are rather limited…

The psychology of foot amputation includes the necessary grieving process to accept the loss, the need to readjust to new sensations and mobility, and the management of emotions such as anxiety or depression. Psychological support, guidance from medical teams, and support groups are crucial for navigating this process and adapting to a new identity and a new body.

The loss of a loved one is similar to the loss of a loved one, or even to one's own death, which is why we speak of a grieving process.

Chapter 1 It's about "living with it."

The work of mourning is long and emotionally demanding. You may experience all sorts of emotions. But know that there is no right or wrong reaction. Each person is unique and reacts in their own way. What you feel and express will gradually allow you to let go of who you were "before" and envision who you want to be "now."

- *First, denial, then shock.*

You minimize or ignore this painful reality, which generates profound anguish. You tell yourself, "No, it's not possible."

- *The time for anger and revolt.*

Protest, even rage, follows denial. You express your suffering through irritability, intolerance, and aggression toward your loved ones and caregivers.

This emotion corresponds to the attempt to return to your previous physical state. It is all the more intense because the prosthetic fitting, often difficult due to a fragile stump, only serves to confront you with the painful reality of loss.

- Depression

Depression often follows irritation. It comes as a form of healing. It's an awareness of the irreversible nature of the loss. It allows you to surrender to sadness, to mourn what you have lost.

- Bargaining or negotiation

This is an attempt to escape the situation or delay the unfolding of events that seem insurmountable. You create a distance between yourself and the daily experience of disability. These are internal negotiations aimed at changing the course of reality. They correspond to the regaining of hope.

- Finally, acceptance

This is the beginning of the process: you realize the importance the prosthesis will have in your life. Gradually letting go of your old body image and your previous life allows you to envision a new relationship with things and people, and to invest in the future. The amputation of a limb will lead to changes that must be accepted in many areas of daily life: work, sports, leisure, and your emotional and social life. It's about rethinking your relationship with your new body image, your new abilities and limitations, and your new status as a "disabled person."

The psychological dimension also encompasses the way others see us: not just those close to us, but those we might encounter every day, in the street, at work, in shops, or in our former collective lives. And this works both ways: the way others perceive the amputee and disability in general; and the way the amputee receives in return, which they accept to varying degrees. It's not simple, especially in a culture obsessed with appearances. Just as the amputee must go through the stages mentioned above, those around them, even the most distant, must also go through stages of acceptance. They can also turn away. A look can help, but it can also symbolically kill. An amputee is not just an individual; they are an issue for their entire environment, even for society as a whole. The ADEPA association, among others, offers opportunities to address these issues, thanks to the shared experience its members can share. The following pages also address these issues at the level of close relatives.

Your family and loved ones are also deeply affected by your amputation: the grieving process applies to everyone around you. During your hospitalization and rehabilitation, you are no longer involved in your family's daily concerns.Friends may be inclined to protect you, treat you like a child, avoid you, or even create a rift. They, too, have their own path to acceptance.

However, they can help you envision life outside the hospital setting, and you can help them as well. The daily struggles your loved ones share with you, and the discussions you have about finding solutions together for adapting to life at home, will allow everyone to consider how to "live with" this new body.

Amputation triggers numerous psychological processes in the person who undergoes it. Psychological support offered in specialized facilities complements rehabilitation, helping the individual to overcome shock and emotional distress, cope with trauma, and regain their place in daily life.

A Matter of the Body

Far more than a mere shell, the body is a whole: it doesn't simply appear to us as a shape, a bodily schema. The body speaks to the brain, and the brain interacts with the body. For example, it frequently happens that a patient "forgets" their amputation and falls when standing up: the body has changed, but the information has not yet been fully integrated by the brain, or perhaps it is a form of denial of reality. It will take time, sometimes a long time, for recognition and acceptance.

The Trauma of Amputation

Amputation is a brutal loss of this illusion of immortality that is constitutive of the human psyche. The amputee has, quite literally, "put one foot in the grave." Moreover, some patients are concerned about what has become of their amputated limb.

Specific Mechanisms and Symptomatology

Amputation, whether the result of an accident or a specific pathology, triggers several mechanisms. Some are observed almost systematically in these patients: a mixture of grief and traumatic disruption. Initially, amputees seem stunned, in shock, as if psychologically numb. This mechanism is observed clinically through asthenia, depression, but also sometimes aggression or euphoria.

In most cases, a renewal will follow.

Rehabilitation and the Patient-Caregiver Relationship

Arrival at a rehabilitation center marks a new stage in the amputee's journey. Desired and driven by the desire to regain autonomy, it is nevertheless often experienced as a trauma. The mirror effect of encountering other amputees leads to a brutal realization of the reality of the world of people with disabilities. Two strategies emerge among patients: either accepting the disability and embracing it, or the destiny.

Psychological Support

Each patient, influenced by their culture, constructs a unique narrative around their amputation: from anger to resignation, including feelings of injustice or guilt; there is always a desire to make sense of the traumatic event. Interviews with the psychologist provide a framework for processing their story and the psychological tensions it entails, as well as for releasing the painful emotions associated with the amputation.

Chapter 2 Most frequent causes/ medical

If you are affected, you are not alone. In Germany, some 30,000 to 40,000 amputations are performed each year, mostly of the foot. Among the main causes of foot amputations are diabetes, specifically diabetic foot syndrome, and serious accidents, such as those at work or in traffic. But obstructive arterial disease, better known as "smoker's leg," also plays a major role.

The number and causes of obstructive arterial disease have remained the same for years. The reason? More and more people are living longer, and consequently, there are more and more people with diabetes mellitus. However, because treatment has improved in recent years, the number of amputations is not increasing. In Germany, more than 20,000 amputations each year are attributable to diabetic foot syndrome.

How is a forefoot amputation performed?

Collaboration between surgeons, internists, chiropractors, physical therapists, and orthotists is essential for an optimal amputation. Because the most important thing for your future life is being able to stand and walk with your foot as normally as possible.

Amputations today are performed according to the following principle: "as much as necessary, but as little as possible." The surgeon, under anesthesia, removes as much tissue as needed to ensure you are as mobile as possible afterward. The doctor makes an incision in the skin, removes the diseased tissue and bone, shapes the stump, and closes the wound. The more healthy tissue that is preserved, the greater the benefits for you.

In principle, the surgeon only attends the pre-operative consultation to explain the procedure for the forefoot amputation. Therefore, they don't know your entire medical history and cannot influence what will happen after the amputation. After the forefoot amputation, you will be cared for by your general practitioner or specialist. Some time later, an orthotist will come to provide you with your prosthetic devices. Together, you will decide which level of amputation is best for your case. The type of amputation has a significant impact on what is possible with your foot after the operation. The surgeon's main role is to perform the amputation in such a way that you can be as active as possible afterward.

Today, the question is no longer how, but precisely where the surgeon begins the amputation and how they shape the stump. This aspect is extremely important, as the stump must then be able to be used without pain and must not be subjected to friction to prevent any soreness. To achieve this, the surgeon "pads" the bone with muscle and leaves enough skin to close the stump without tension. If possible, the scar is placed on the top of the foot to avoid stress and friction.

In the best-case scenario, amputation of one toe is sufficient. In all cases, the surgeon attempts to preserve the entire metatarsal bone. If the head of the toe joint cannot be saved, the corresponding metatarsal bone must also be (partially) removed. Doctors then refer to this as a "ray." The loss of a ray significantly impacts the ability to stand and walk. Without a prosthesis, it leads to postural abnormalities. If all the metatarsal bones are affected, the surgeon must also remove them all. To avoid unequal bone lengths, a rounded stump is created. This also facilitates the subsequent fitting of a custom-made prosthetic foot.

Even if the amputation affects the tarsal bones or even the entire tarsus, the surgeon attempts to create a rounded stump to simplify fitting with a prosthesis. In all these cases, the ankle is preserved. With a custom-made forefoot prosthesis, the foot can generally bear weight again. You can stand and walk. When the ankle is also amputated, but not the knee, it is called a transtibial amputation. When the knee is also involved, it is called a transfemoral amputation. In principle, the surgeon who performs the operation decides on the level of amputation, in consultation with the orthopedic surgeon. However, to prepare for long-term prosthetic fitting, it is helpful to involve the orthotist/prosthetist in this decision prior to the amputation.

Let's be honest: you won't be in great shape after the amputation. You'll have to cope with a significant loss and may experience a loss of self-confidence. You'll need to get used to new bodily sensations.

After a forefoot amputation, your stability changes. Part of the leverage you need to walk is missing. Initially, you'll have difficulty walking and standing. As a precaution, you'll place your foot less firmly on the ground with your stump. You'll likely develop postural abnormalities to compensate. It's therefore crucial to learn about available assistive devices in a timely manner. These devices will help you regain your normal movements.

You might feel ashamed, or perhaps you'll find it difficult to be seen in public with your amputated foot. Prosthetics can help you adapt more quickly to your new situation. A forefoot prosthesis offers freedom of movement in your ankle joint. This freedom will also allow you to perform almost natural movements.

Your biggest challenge will likely be maintaining a social life and getting used to your new bodily sensations. You have every reason to be confident, though. After all, who can boast of having gone through what you have?

As soon as your forefoot amputation takes place, you'll begin the journey back to your normal life. To quickly regain your ability to walk, cycle, or drive, the wound must heal properly and your muscles must regain strength. This requires both strength and patience. The first few weeks are crucial for how you will manage your life with your amputated limb.

After the amputation, you will initially need to remain lying down for a few days, avoiding putting any weight on your foot. A medical team will be there to support you very soon. Rehabilitation can then begin. No one can tell you in advance how long it will take for the wound to heal or how long it will take for you to walk again, drive, or return to work. However, experience shows that all doctors will tell you that the more active and positive you are, the faster you will progress during rehabilitation. Initially, all efforts are focused on healing. As soon as the healing process begins, you will work on your mobility with a physiotherapist to ensure your muscles remain strong and your joints remain mobile despite the long period of inactivity. Later, they will guide you through specific movements and strengthen the muscles in your residual limb so you can walk safely with a prosthesis.

The physiotherapist has another important role: to help you train yourself to feel your foot stump. Your brain isn't used to your stump and, initially, can't process the nerve signals. However, it's essential for your health that you accurately feel your foot. Once the wound has completely healed and you can put weight on the stump, you'll be fitted with a prosthesis, which you'll need to gradually get used to.

During a transition phase, you may receive a forefoot offloading shoe. This allows you to walk without putting too much weight on the wound. As part of your rehabilitation, you will receive occupational therapy. The occupational therapist will help you improve your dexterity and perform movements related to daily life and your work. Their support will help you better control everyday movements.

Rehabilitation also includes participation in gait training, guidance from a professional coach, a psychologist, and a family counselor. Your rehabilitation also involves analyzing your lifestyle: Is your diet healthy? Do you smoke? If you manage to overcome an amputation, you can also quit smoking. Want to bet?

After your forefoot amputation, you must initially remain lying down with your foot elevated to allow blood to return to your heart. During the operation, the blood and interstitial fluid that accumulate around the wound must be gradually drained.

An elastic bandage facilitates this drainage. It applies even pressure without opening the wound. The wound is checked daily for any inflammation or congestion, and the bandage is also changed daily. The healing process is therefore continuously monitored by specialized staff. The bandage plays another important role: it shapes the stump so that it can later bear weight. This is why the bandage should always be applied by professionals. It's important to remember that a forefoot amputation is a major procedure and the wound is considered severe. Complete healing can take weeks or even months. Wound healing can be accompanied by complications, as is often the case with patients suffering from diabetes. When diabetes is the cause of an amputation, diabetic foot syndrome also hinders healing. Time is your best ally at this stage.

In the case of smaller amputations, custom-made insoles may suffice. An orthotic insole is not intended to restore function, but in the case of much less extensive surgery, it is sufficient in certain circumstances to stabilize the foot.

A silicone forefoot prosthesis allows for free ankle movement and is individually fitted. Silicone forefoot prostheses are individually and specifically adapted to your residual limb by the orthotist. They fit like a glove. Ankle movements are unrestricted. Your foot is fully mobile. All movements feel natural. Recent studies demonstrate that the muscles are also more active than with other prostheses. Thanks to the flexible material, it is possible to walk longer distances and wear regular shoes.

Some doctors and orthotists recommend custom-made shoes crafted by an orthopedic shoemaker. However, please ensure that this is truly the best option for you. Not only is it rather conspicuous, but it can also present drawbacks since ankle movement is restricted. Walking in this type of shoe is not always very flexible and can be uncomfortable. Orthopedic insoles, also made by an orthopedic shoemaker, are more discreet. This version also supports the ankle, so you cannot wear standard shoes.

The Bellmann prosthesis, made by an orthotist, allows for greater mobility. Using a sock, you put on a prosthesis composed of several layers of foam. This device does not cover the top of the ankle joint. It offers other advantages such as its light weight and shape, which allow you to wear ready-made shoes. However, the appearance of the Bellmann prosthesis cannot replicate that of your other foot. It is therefore clearly visible as a prosthesis. Furthermore, its material wears out very quickly. After two years, you will need a new prosthesis, and the sock can cause friction, which can be bothersome.

The Different Types of Foot Amputations

Not all types of trauma will require similar surgical interventions.

In fact, an amputation is named according to the type of cut it involves.

Below are listed the surgical amputation procedures that affect the foot or part of the foot:

- Transmetatarsal amputation: This operation aims to remove a portion of the forefoot from the middle of the metatarsals.

- Chopart amputation: Although rarely performed, this type of surgery involves the removal of the front part of the talus and calcaneus.
- Lisfranc amputation: This involves the removal of all the toes and metatarsal bones.
- Syme amputation: This surgical procedure allows for the complete removal of the foot by incising the joint between the leg and the foot.
- Following any of these procedures, the doctor may recommend the fabrication of a prosthesis or the use of a specialized orthotic device. Complications of a Foot Amputation

Obviously, this treatment has significant repercussions on the body and the patient's overall quality of life. Physiotherapy sessions are necessary to become familiar with the prosthesis or the partially amputated foot.

At the same time, a newly amputated person should remain attentive to the appearance of symptoms that could become problematic:

1. Pain in the stump
2. Dry skin
3. Skin that appears inflamed
4. Foul-smelling fluid discharge from the wound
5. Opening of the surgically closed wound
6. Excessive sweating near the stump
7. Bruising or redness on the stump

A "phantom limb" sensation, where the missing limb still seems to be present

If one or more of these signs are identified, it is recommended to consult a healthcare professional to prevent potential complications. Such a device will compensate for some of the foot functions lost during the amputation.

Conclusion

Amputation imposes transformations and adjustments on patients on psychological, physical, familial, social, and professional levels. From the trauma to the ordeal of reconstruction, it is a singular and challenging event. Psychological support in a rehabilitation center is therefore conceived as an attempt to temporarily act as a container for the overwhelming excitation within the patient's psyche, until they recover.

Bibliography:

1. Aiordăchioae, Gigi Adrian, Implicaţiile protezării în dinamica articulară şi în menţinerea sanogenezei la amputaţiile membrului inferior pentru boala arterială periferică, Editura Universitatea de Vest "Vasile Goldiş" din Arad. Facultatea de Medicină, Arad, 2013
2. Antakjy, Kussay Ahmed Subhi, Tribological Study of Human Skin in Exoprostheses for Lower Limb Amputation, Bucharest, 2018
3. Ham, R., Limb amputation, Ed. Cambridge, London, 1991
4. Nedea, Mărioara, Vis in roz, vitalitate, Amputare, Ed. Transversal, Bucuresti, 2020

YOGA AND THE RESPIRATORY APPARATUS

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DOI: https://doi.org/10.66793/titum19proceeding7

Abstract

Yoga used is based on the structure and function of the human body. Because yoga practice emphasizes the relationship of the breath and the spine, it is a particular attention to those systems. . Prana refers not only to what is brought in as nourishment but also to the action that brings it in.

The yogic concept that complements prana is apana, which refers to what is eliminated by a living thing as well as the action of elimination. These two fundamental yogic terms—prana and apana—describe the essential activities of life.

The diaphragm is the prime mover of the thoracic and abdominal cavities. The specific patterns that arise in yoga asana practice or breathing exercises, however, result from the action of muscles other than the diaphragm that can change the shape of the cavities. These are called accessory muscles. The analogy of a car and its engine is very useful in explaining this principle.

Asanas are body positions (postures) specific to the practice of yoga. Originally, the term referred to a seated meditation position, but in modern yoga, it includes any type of body posture, such as standing, lying, inverted, twisted, or balancing. They are fundamental elements of yoga practice, contributing to the promotion of health This work presents several asanas in which a study of both respiration and motility is made. The five "usual positions" are commonly referred to as the starting positions.

Standing—supported on the soles of your feet
Sitting—supported on the base of your pelvis
Kneeling—supported on your knees, shins, and tops of feet
Supine—supported on the back surface of your body
Prone—supported on the front surface of your body

Keywords
Yoga, prana, apana, diaphragm,asanas

The view of yoga used is based on the structure and function of the human body. Because yoga practice emphasizes the relationship of the breath and the spine, it is a particular attention to those systems. By viewing all the other body structures in light of their relationship to the breath and spine, yoga becomes the integrating principle for the study of anatomy. Additionally, for yoga tool for keeping our bodies safe and our minds grounded in reality.

The reason for this mutually illuminating relationship between yoga and anatomy is simple: The deepest principles of yoga are based on a subtle and profound appreciation of how the human system is constructed. The subject of the study of yoga is the Self, and the Self is dwelling in a physical body.

The context that yoga provides for the study of anatomy is rooted in the exploration of how the life force expresses itself through the movements of the body, breath, and mind.

A key element that distinguishes yoga practice from gymnastics or calisthenics is the intentional integration of breath, posture, and movement. The essential yogic concepts that refer to these elements are beautifully expressed b

prana/apana
sthira/sukha
sukha/dukkha

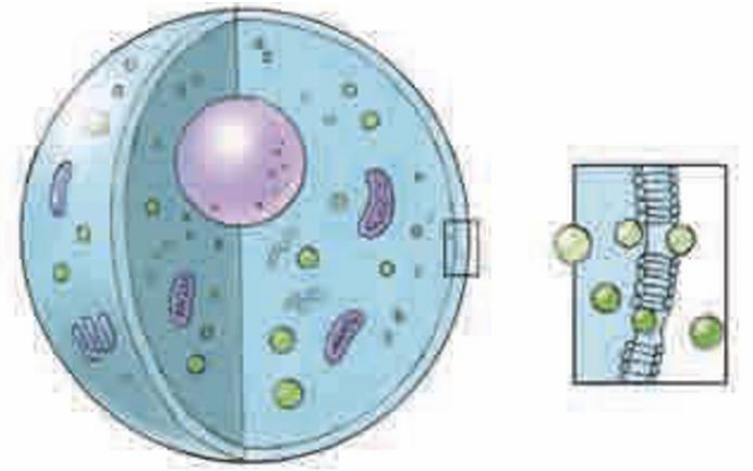
Prana and apana

A cell consists of three parts: the cell membrane, the nucleus, and the cytoplasm. The membrane separates the cell's external environment, which contains nutrients that the cell requires, from its internal environment, which consists of the cytoplasm and the nucleus. Nutrients have to get through the membrane, and once inside, the cell metabolizes these nutrients and turns them into the energy that fuels its life functions. As a result of this metabolic activity, waste gets generated that must somehow get back out through the membrane.

This observation that living things take in nutrients provides a good basis for understanding the term prana, which refers to what nourishes a living thing. Prana refers not only to what is brought in as nourishment but also to the action that brings it in.

The yogic concept that complements prana is apana, which refers to what is eliminated by a living thing as well as the action of elimination. These two fundamental yogic terms—prana and apana—describe the essential activities of life.

Successful function, of course, expresses itself in a particular form. Certain conditions have to exist in a cell for nutrition (prana) to enter and waste (apana) to exit. The membrane's structure has to allow things to pass in and out of it—it has to be permeable.



1.The cell's membrane must balance containment (stability) with permeability.

Sukha and Dukha

The pathways must be clear of obstructing forces in order for prana and apana to have a healthy relationship. In yogic language, this region must be in a state of sukha, which literally translates as "good space." "Bad space" is referred to as dukha, which is commonly translated as "suffering."

The basic idea is that when you make more "good space," your pranic forces will flow freely and restore normal function. This is in contrast to any model that views the body as missing something essential, which has to be added from the outside.

Breathing, Gravity, and Yoga

In utero, oxygen is delivered through the umbilical cord. The mother does the breathing. There is no air and very little blood in the lungs when in utero because the lungs are non functional and mostly collapsed. The circulatory system is largely reversed, with oxygen-rich blood flowing through the veins and oxygen-depleted blood flowing through the arteries. Humans even have blood flowing through vessels that won't exist after birth, because they will seal off and become ligaments.

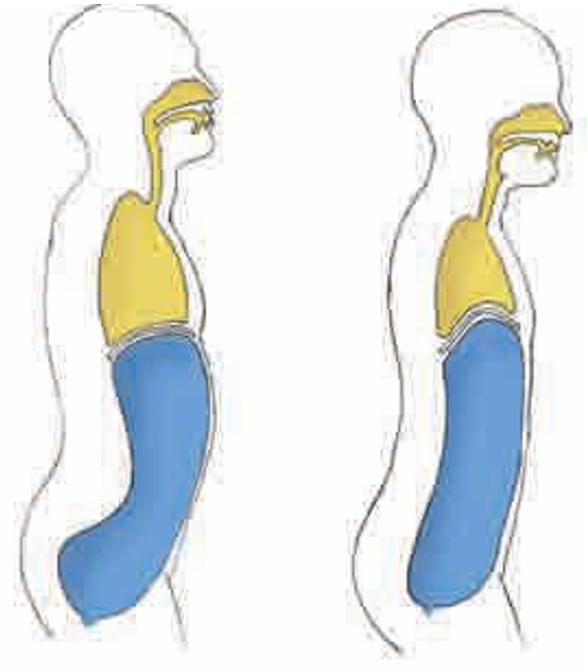
That first inhalation was the most important one because the initial inflation of the lungs causes essential changes to the entire circulatory system, which had previously been geared toward receiving oxygenated blood from the mother. The first breath causes blood to surge into the lungs, the right and left sides of the heart to separate into two pumps, and the specialized vessels of fetal circulation to shut down and seal off.

That first inhalation is the most forceful one you will ever take because it needs to over come the initial surface

tension of your previously collapsed and amniotic-fluid-filled lung tissue. The force required (called negative inspiratory force) is three to four times greater than that of a normal inhalation.

Movement in Two Cavities

These cavities share some properties, and they have important distinctions as well. Both contain vital organs: Another important shared property of the two cavities is that they are mobile—they change shape. It is this shape-changing ability that is most relevant to breathing, because without this movement, the body cannot breathe at all. Although both the abdominal and thoracic cavities change shape, there is an important structural difference in how they do so.

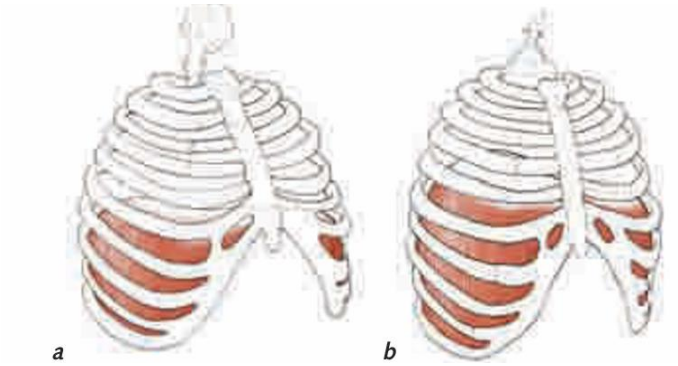


2.Breathing is thoracoabdominal shape change. Inhalation on left, exhalation on right.

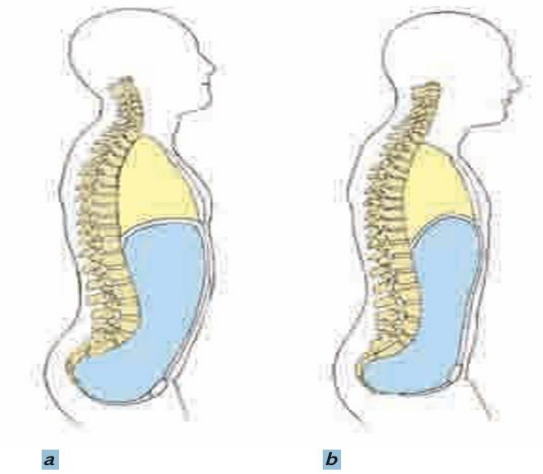
The thoracic and abdominal cavities as an accordion stacked on top of a water balloon. This illustration gives a sense of the relationship of the two cavities in breathing; movement in one will necessarily result in movement in the other. Recall that during an inhalation (the shape change permitting air to be pushed into the lungs by the planet's atmospheric pressure), the thoracic cavity expands its volume. This pushes downward on the abdominal cavity, which changes shape as a result of the pressure from above.

Three-Dimensional Shape Changes of Breathing

Because the lungs occupy a three-dimensional space in the thoracic cavity, when this space changes shape to cause air movement, it changes shape three-dimensionally.



3.Three-dimensional thoracic shape changes of (a) inhalation and (b) exhalation.



4.Changes in abdominal shape during breathing: (a) Inhalation = spinal extension; (b) exhalation = spinal flexion.

Diaphragm's Role in Breathing

The diaphragm is the principal muscle that causes three-dimensional shape change in the thoracic and abdominal cavities.

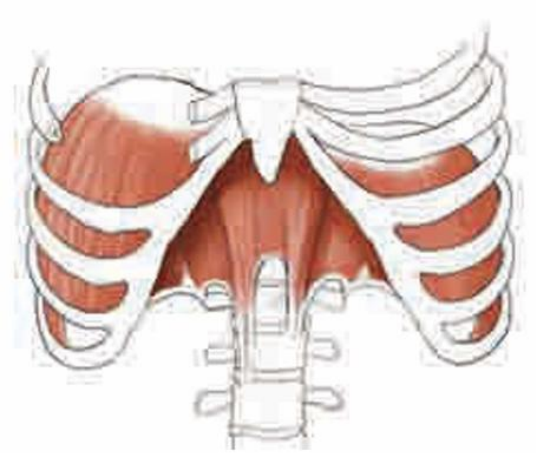
The diaphragm divides the torso into the thoracic and abdominal cavities. It is the floor of the thoracic cavity and the roof of the abdominal cavity. Its structure extends through a wide section of the body—the uppermost part reaches the space between the third and fourth ribs, and its lowest fibers attach to the front of the third lumbar vertebra; "nipple to navel" is one way to describe it.

The lower edges of the diaphragm's circumference originate from three distinct regions: the bottom of the sternum, the base of the rib cage, and the front of the lower spine.

The central tendon of the diaphragm is a point of anchorage for the connective tissue that surrounds the thoracic and abdominal organs. The names of these important structures are easily remembered as the three Ps.

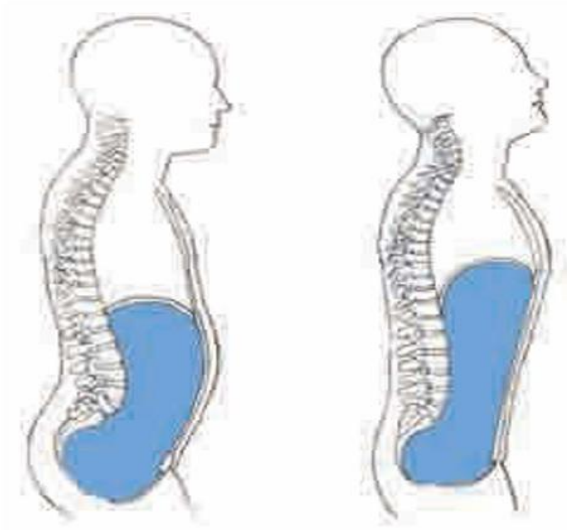
- Pleura, which surround the lungs
- Pericardium, which surrounds the heart
- Peritoneum, which surrounds the abdominal organs

As in any other muscle, the contracting fibers of the diaphragm pull its insertion and origin (the central tendon and the base of the rib cage) toward each other. This muscle action is the fundamental cause of the three-dimensional of breathing.

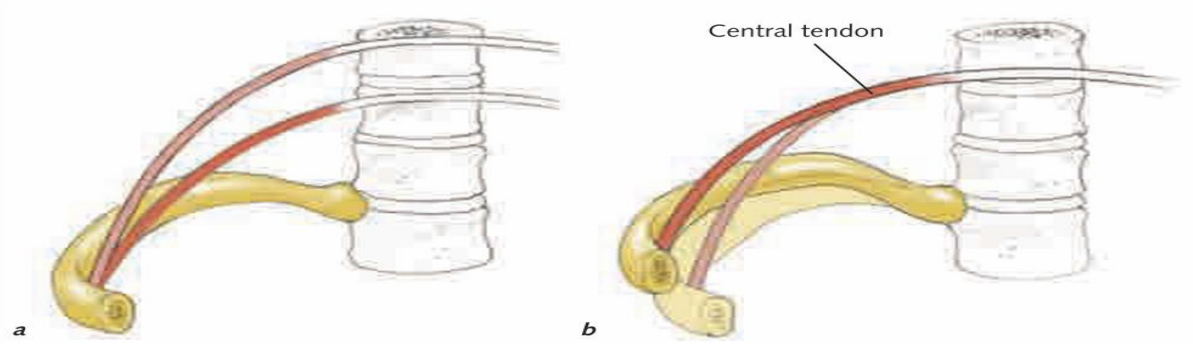


5.The muscle fibers of the diaphragm all run vertically from their origins to their insertion on the central tendon.

If the central tendon is stabilized and the ribs are free to move, a diaphragmatic contraction will cause an expansion of the rib cage . This is a “chest breath,” which many people believe must be caused by the action of muscles other than the diaphragm.



6.The diaphragm can be (a) a “belly bulger,” during the belly inhalation, or (b) a “rib cage lifter,” during the chest inhalation.



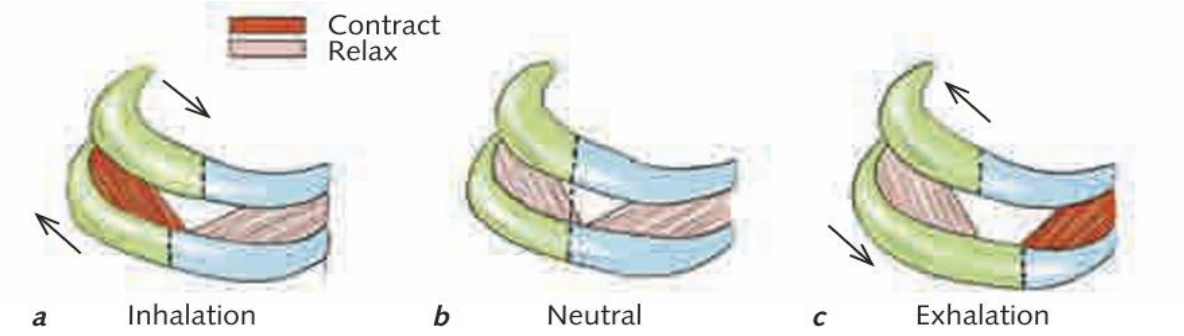
7.(a) With the rib stable and the abdominals relaxed, the diaphragm's contraction lowers the central tendon. (b) With the rib cage relaxed and the central tendon stabilized by abdominal action, the contracting diaphragm lifts the rib upward.

The diaphragm is the prime mover of the thoracic and abdominal cavities. The specific patterns that arise in yoga asana practice or breathing exercises, however, result from the action of muscles other than the diaphragm that can change the shape of the cavities. These are called accessory muscles. The analogy of a car and its engine is very useful in explaining this principle.

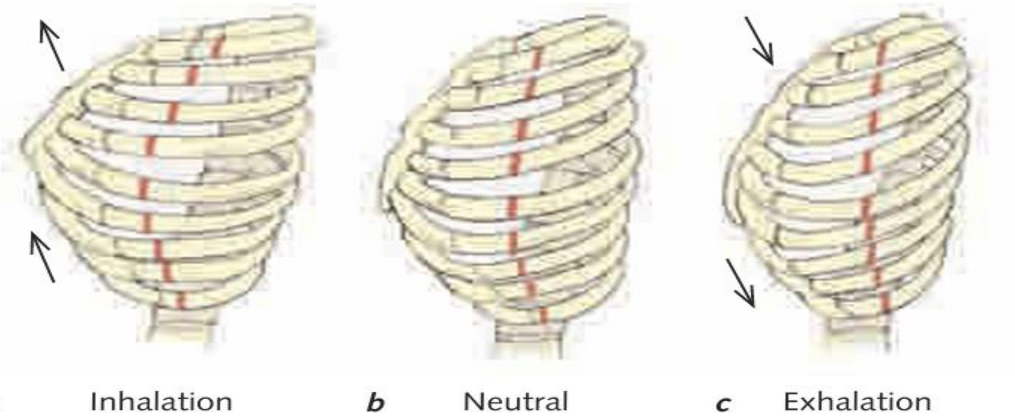
Additionally, the notion that that diaphragmatic action is limited to abdominal bulging (belly breathing) is as inaccurate as asserting that a car's engine is only capable of making it go forward—and that some other source of power must govern reverse movement. Just as this automotive error is linked to not understanding the relationship of the car's engine to its transmission, the breathing error results from not understanding the relationship of the diaphragm to the accessory muscles.

Accessory Muscles of Respiration

These muscles are universally classified as "exhaling muscles," but here they actively participate in shaping an inhalation. In the chest breath, the central tendon (insertion) of the diaphragm is stabilized by the abdominal muscles, also regarded as "exhaling muscles," but in this case, they are clearly acting to produce a pattern of inhaling.



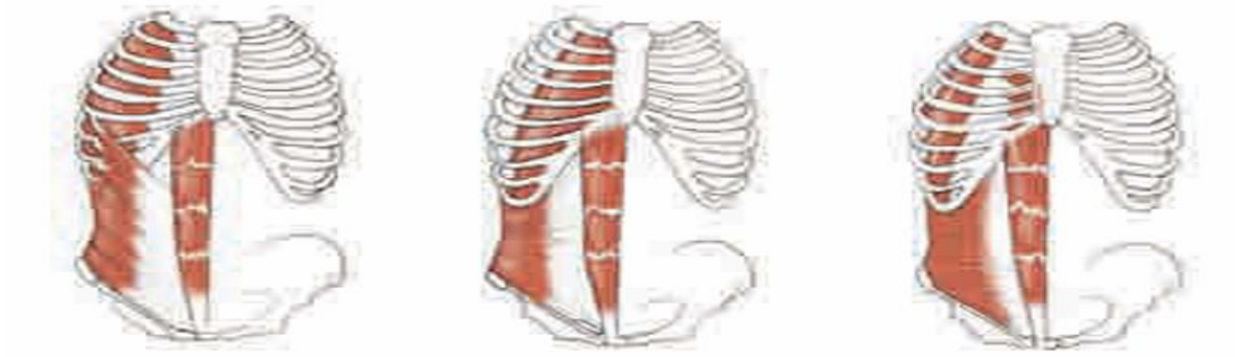
8.The intercostal muscles assist the sliding action of the ribs during respiration. During inhalation (a), the external intercostals contract, and the internal intercostals relax. During exhalation (c), the opposite occurs.



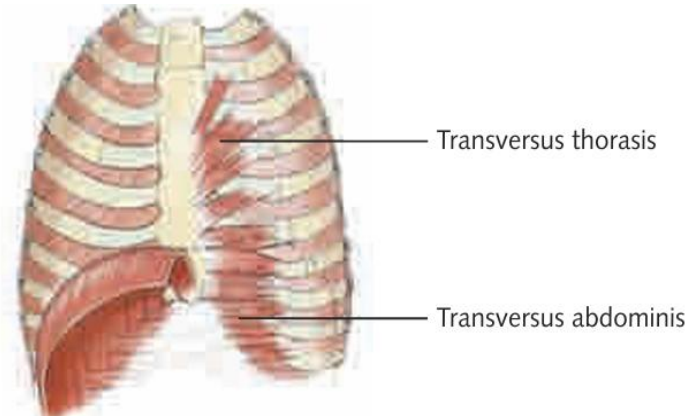
9.Contrary to appearances, the intercostal spaces remain constant during respiratory movements. Rather, the ribs slide in relation to each other—as indicated by the misaligning of the red line

Abdominal and Thoracic Accessory Muscles

The abdominal cavity and its musculature can be imagined as a water balloon surrounded on all sides by elastic fibers running in all directions**.** The abdominal muscles that have the most direct effect on breathing are the ones that originate at the same place as the diaphragm, the transversus abdominis. The transversus abdominis the direct antagonist to the diaphragm's action of expanding the rib cage. The same layer of horizontal fibers extends upward into the posterior thoracic wall as the tranversus thoracis, a depressor of the sternum.



10.The continuity of the abdominal and intercostal layers shows how the external obliques turn into the external intercostals, internal obliques turn into the internal intercostals, and the transversus abdominis turns into the transversus thoracis and innermost intercostals.



11.Posterior view of the chest wall, showing the interdigitated origins of the diaphragm and transversus abdominis forming perfect right angles with each other. This is clearly an agonist/antagonist, inhale/exhale muscle pairing that structurally underlies the yogic concepts of prana/apana.
Chest, neck, and back muscles can expand the rib cage , but they are far more inefficient than the diaphragm and external intercostals at doing this. This inefficiency is the result of the fact that the location and attachment of these muscles do not provide enough leverage on the rib cage, and the usual role of these muscles is not respiration.

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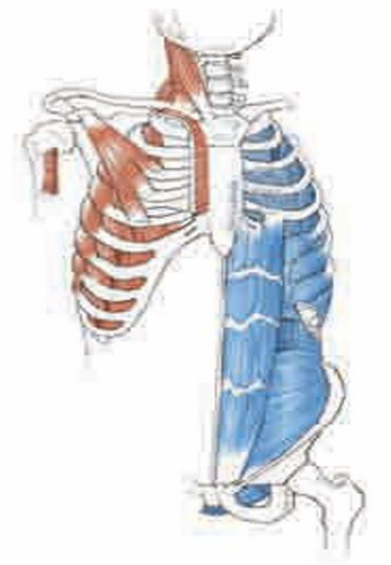
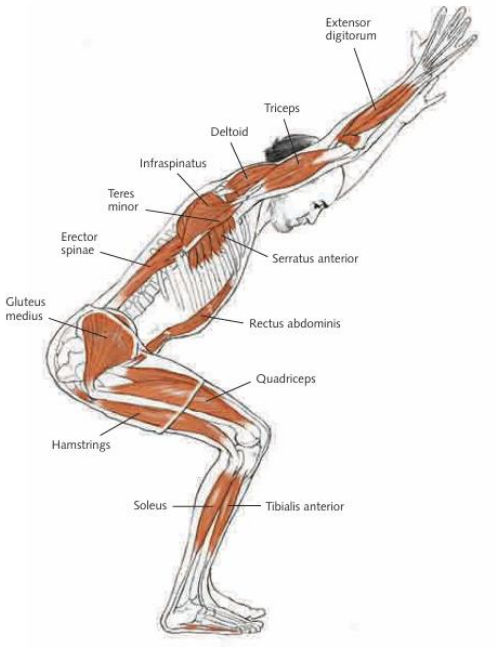


12. Some of the accessory muscles of respiration: Blue muscles act to reduce thoracic volume, while red muscles help to increase thoracic volume
Along with the respiratory diaphragm, breathing involves the coordinated action of the pelvic and vocal diaphragms. Of particular interest to yoga practitioners is the action of mula bandha, which is a lifting action produced in the pelvic floor muscles that also includes the lower fibers of the deep abdominal layers. Mula bandha is an action that moves apana upward and stabilizes the central tendon of the diaphragm. Inhaling while this bandha is active requires a release of the attachments of the upper abdominal wall, which permits the diaphragm to lift the base of the rib cage upward.

Understanding the asanas
Asanas are body positions (postures) specific to the practice of yoga. Originally, the term referred to a seated meditation position, but in modern yoga, it includes any type of body posture, such as standing, lying, inverted, twisted, or balancing. They are fundamental elements of yoga practice, contributing to the promotion of health
Deciding which anatomical details of yoga poses to depict is quite a challenge. Unlike weight training and stretching, which focus on specific muscles, yoga focuses on asanas that are whole-body practices; no elements are entirely passive.
The five “usual positions” are commonly referred to as the starting positions. Any asana you can think of has one of these common positions as its starting point:
Standing—supported on the soles of your feet
Sitting—supported on the base of your pelvis
Kneeling—supported on your knees, shins, and tops of feet
Supine—supported on the back surface of your body
Prone—supported on the front surface of your body
Types of Muscle Contractions
Concentric—The length of the muscle decreases during a contraction.

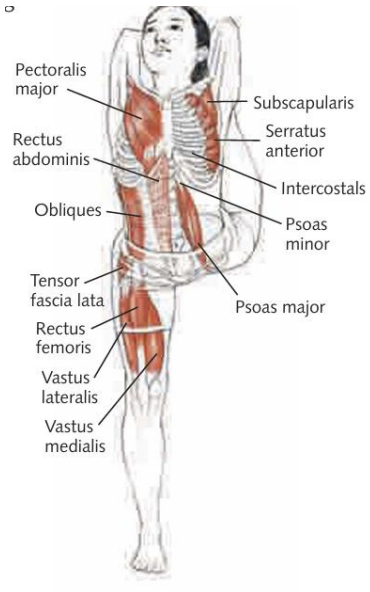
Eccentric—The length of the muscle increases during a contraction.
Isometric—The length of the muscle remains constant during a contraction against resistance, and the intention is to not move.
Isotonic—The length of the muscle remains constant during a contraction against resistance, and the intention is to move.

Standing poses
Utkatasana Chair Pose



13. Key Structures Shoulder girdle, spine, quadriceps and hamstrings to balance each other, knees (adductors and medial rotators). To protect knees, minimize external rotation as the hips flex.
Breathing Maintaining axial extension (which minimizes breathing shape change) while engaging the largest, most oxygen-hungry muscles of the body presents a challenge that requires efficiency of effort and breath. Otherwise, the body's oxygen demands will make the breath too labored to continue to maintain the axial extension.

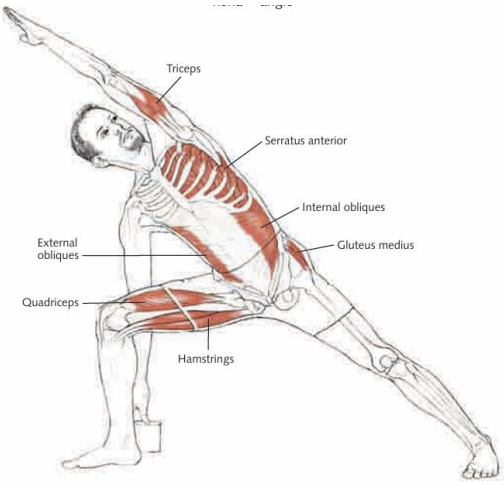
Natarajasana King of the Dancers Pose



14.Joint Actions Spinal extension; scapula upward rotation, abduction, and elevation; arm flexion; elbow flexion; and forearm supination. Standing leg: hip flexion, knee extension, and ankle dorsiflexion. Lifted leg: hip extension, knee flexion, and ankle plantarflexion.

Breathing The excursion of the diaphragm is greatly minimized in dancer's pose by the combination of deep spinal extension and the anterior and posterior musculature working against each other to stabilize this shape in gravity. Consequently, this pose should be held with quiet breathing, and seldom for a very long time, because the muscular effort required to maintain it soon outpaces the body's ability to supply those muscles with oxygen.

Utthita Parsvakonasana Extended Side Angle Pose

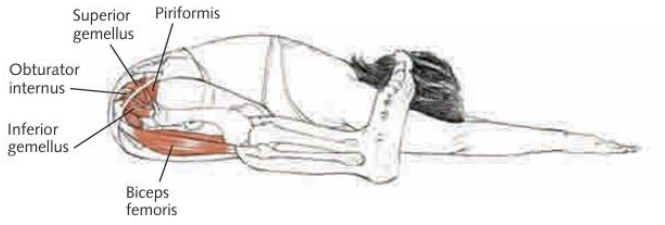


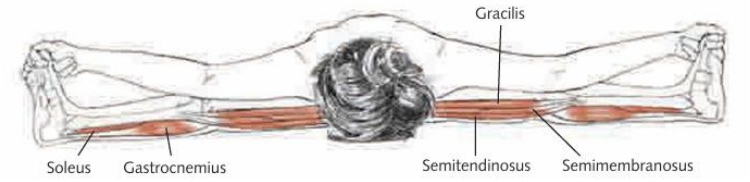
15. Joint Actions Gluteus medius Spine neutral or with slight lateral flexion; shoulder abduction, upward rotation; glenohumeral joint flexion and external rotation; elbow extension. Front leg: nutation; hip flexion, external rotation, abduction; knee flexion; ankle dorsiflexion.

Breathing Even though the upper side of the breathing mechanism receives a strong stretch in this shape, the more interesting effect may be on the lower side of the body, where the dome of the diaphragm is driven cranially by the force of gravity acting on the abdominal organs. Breath action in this position provides very useful asymmetrical stimulation to the diaphragm and all the organs attaching to it.

Sitting poses

Upavistha Konasana Seated Wide-Angle Pose

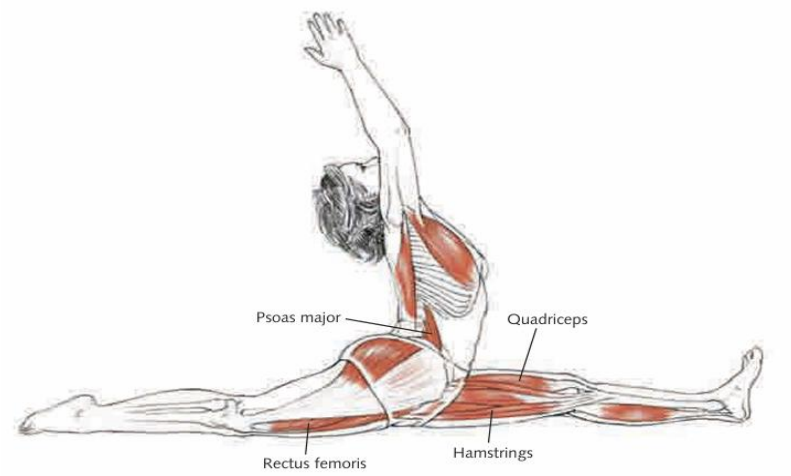




16.Joint Actions Semitendinosus Semimembranosus Mild spinal flexion (moving toward axial extension); sacrum nutation; major hip abduction, external rotation, and flexion; knee extension; ankle dorsif lexion.

Breathing The act of gradually lengthening the spine in this pose can be greatly assisted by the breath. The exhalation, if initiated in the lower abdomen, can help anchor the sitting bones and ground the backs of the thighs, whereas the inhalation, if it's initiated in the upper chest, can help to lengthen the spine. In short, the exhalation can ground the posture's lower half, and the inhalation can lengthen the posture's upper half.

Hanumanasana Monkey Pos

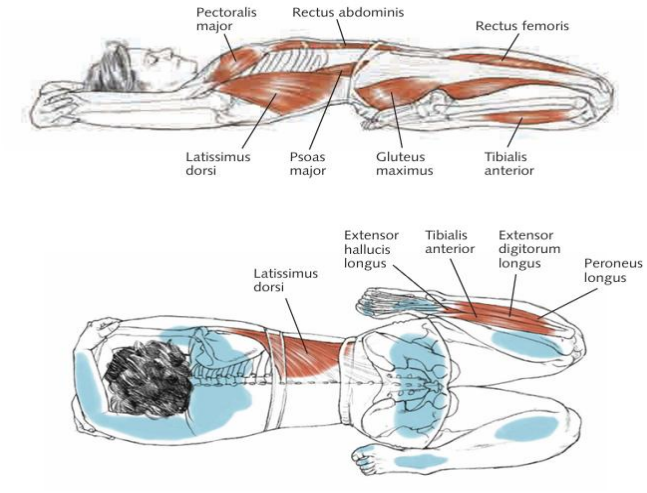


17.Joint Actions Spinal extension. Front leg: sacrum nutation; hip flexion, internal rotation, adduction; knee extension; ankle neutral extension. Back leg: sacrum counternutation; hip extension, internal rotation, adduction; knee extension; ankle plantarflexion; scapula upward rotation, abduction, elevation; glenohumeral joint flexion, adduction, external rotation; elbow extension; forearm neutral.

Breathing You can breathe freely. Until all the flexion, extension, and rotational forces have been neutralized, and the spine can extend easily, the breathing will tend to be labored and rough. The use of props such as blocks, straps, or blankets is highly recommended so that the work can be done in a gradual way that doesn't excessively disturb the rhythm of the breath.

Kneeling poses

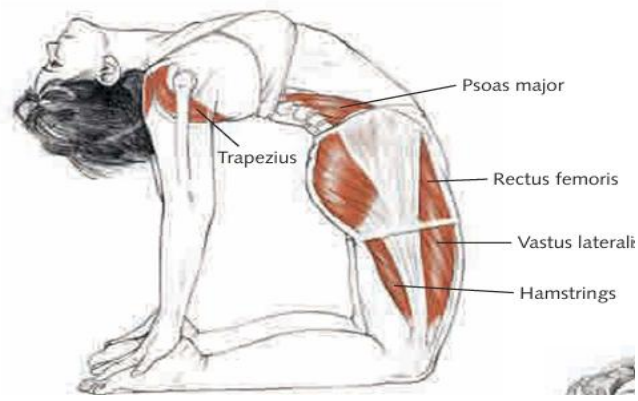
Supta Virasana Reclining Hero Pos



18. Joint Actions Peroneus longus Spine axial extension (in the full version of the pose); sacroiliac joint counternutation; hip extension, internal rotation, and adduction; knee flexion and tibia medially rotated; ankle plantarflexion; scapula upward rotation, abduction, elevation; glenohumeral joint flexion, external rotation; elbow lexion.

Breathing The tautness in the psoas and abdominal wall creates both posterior and anterior pressure in the abdominal cavity. This effect is magnified when activating the abdominal muscles to flatten the lumbar curve. The resulting breathing patterns would favor movements above and below the abdominal pressure. Emphasizing thoracic breath movements at the base of the rib cage helps to mobilize the upper spine and shoulder girdle. Focusing on pelvic floor movements assists in releasing tension in the hips, groin, and gluteal region.

Ustrasana Basic kneeling backbend

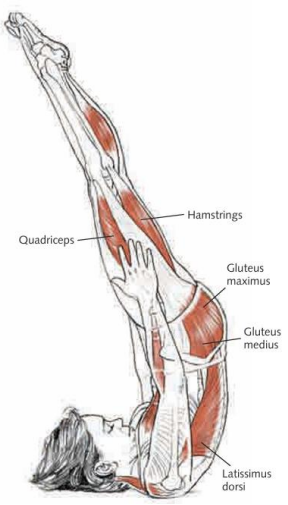


19.Joint Actions Spinal extension; hip extension and internal rotation; knee extension; scapula downward rotation, adduction, elevation; arm external rotation, extension, adduction; elbow extension

Breathing In ustrasana, the thoracic structures are maintained in an “inhaled” position, and the abdominal wall is stretched. This results in a decreased ability of the body to breathe “normally.” The trick is to find support from the deeper musculature so the more superficial efforts can quiet down. Then it’s possible to notice an interesting relationship between the deepest layer of superficial neck muscles (scalenes) and the breath movement in the apex of the lungs, which are suspended from the inner scalene muscles.

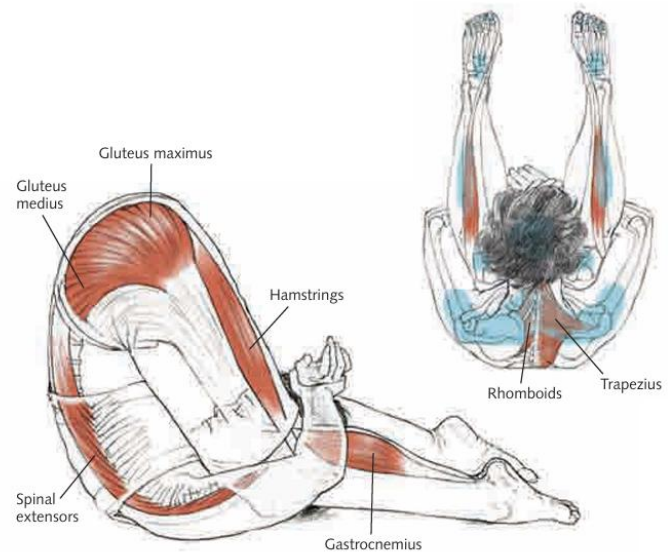
Supine poses

Niralamba Sarvangasana Intermediate supine inversio



20.Joint Actions Spine: Same as described in salamba sarvangasana. Legs: Same as salamba sarvangasana. Arms: Scapula adduction and upward rotation, elevation; glenohumeral joint external rotation, neutral flexion, Breathing In niralamba sarvangasana, the intense action in the full-body's flexor and extensor groups creates quite a challenge to the shape change of breathing. Because this is a challenging balance pose that requires a lot of stabilizing action in the abdominal and thoracic musculature, any attempt at deep breathing will destabilize the pose even as the full-body activation of these major muscle groups creates a demand for significant oxygenation

Karnapidasana Intermediate inverted forward bend



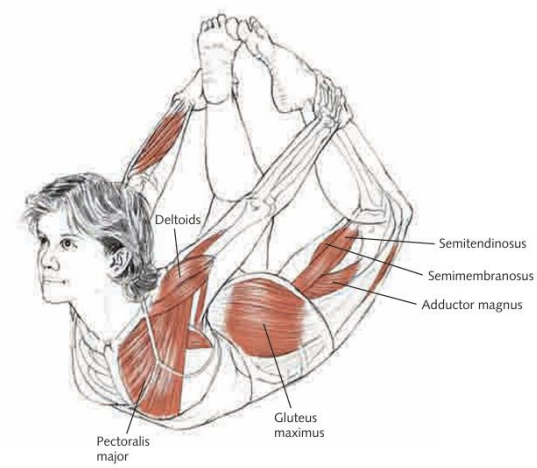
21.Joint Actions Rhomboids Trapezius Spinal flexion, hip and knee flexion, scapula abduction and upward rotation, arm flexion, elbow flexion

Breathing In ear-to-knee pose, the weight of the lower body is bearing down into the torso, which is in maximal flexion—this is basically, an inverted, weight-bearing exhalation.The restriction that this position imposes on the breath shouldn't be a problem as long as the body is flexible enough to be in repose. If the muscles are tense,

the limited capacity to breathe will soon result in the muscles' inability to fuel their activity; at this point, the asana should be exited

Prone poses

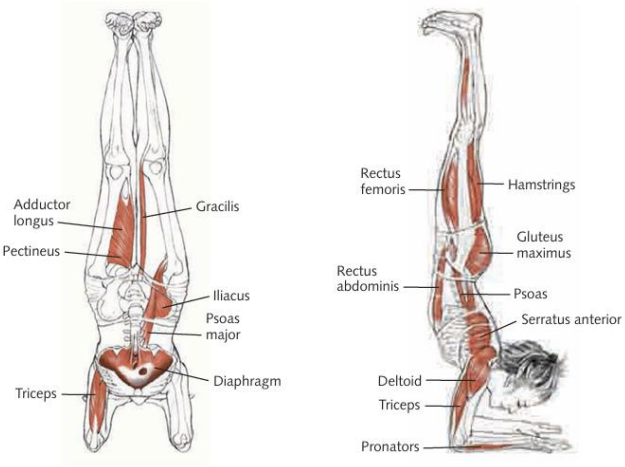
Dhanurasana Basic or intermediate prone backbend



22.Joint Actions Gluteus maximus Semitendinosus Semimembranosus Adductor magnus Spine extension; sacrum counternutation; hip extension, medial rotation, adduction; knee flexion; ankle plantarflexion; scapula adduction, elevation; glenohumeral joint medial rotation, extension, adduction; elbow extension; forearm pronation; finger and hand flexion.

Breathing It is a common practice to rock back and forth in this pose by pushing the belly into the floor with each inhalation. It is less common (but much more intense) to practice not rocking by directing the inhalation into the already expanded chest region

Pincha Mayurasana Advanced inverted arm support



23.Joint Actions Serratus anteriorExtension is maintained throughout spine: The more extension there is in the thoracic spine, the less there will have to be in the cervical and lumbar spines. Neutral hip extension; knee extension; ankle neutral dorsiflexion; scapula abduction, upward rotation, and elevation; glenohumeral joint flexion, external rotation, and adduction; elbow flexion; and forearm pronation.

Breathing The base of support for this pose is formed by the forearms, rib cage, and thoracic spine, and these structures need to be quite stable to maintain balance. Because of this, excessive chest breathing interferes with

supporting a forearm stand. On the other hand, the weight of the legs and pelvis and the curve of the lumbar spine need to be stabilized by the abdominal muscles, making too much abdominal movement counterproductive. Because of these factors, a breathing pattern that moves equally and smoothly throughout the body is needed.

References

1.Adler, S.S., D. Beckers, and M. Buck. 2003. PNF in Practice. 2nd ed. New York: Springer.
2.Clemente, C.D. 1997. Anatomy: A Regional Atlas of the Human Body. 4th ed. Philadelphia, PA: Lippincott Williams & Wilkins.
3.Gorman, David. 1995. The Body Moveable. 4th ed. Guelph, Ontario: Ampersand Press, 1995.
4./ Kapit, W., and L.M. Elson. 1993. The Anatomy Coloring Book. 2nd ed. New York: HarperCollins College Publishers.
5. Kendall, F.P., E.K. McCreary, and P.G. Provance. 1993. Muscles, Testing and Function. 4th ed. Philadelphia, PA: Lippincott Williams & Wilkins.
6.Laban, R. 1966. The Language of Movement: A Guidebook to Choreutics. Great Britain: Macdonald and Evans.
7. Myers, Tom. 2001. Anatomy Trains: Myofascial Meridians for Manual and Movement Therapists. Churchill Livingstone.
8. Netter, F.H. 1997. Atlas of Human Anatomy. 2nd ed. East Hanover, NJ: Novartis.
9/ Platzer, W. 2004. Color Atlas and Textbook of Human Anatomy. Volume 1: Locomotor System. 5th ed. New York: Thieme.

PRIMARY SYNOVIAL NEOPLASM

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DOI: https://doi.org/10.66793/titum19proceeding8

ABSTRACT

Primary pulmonary synovial sarcoma is rare and presents a diagnostic challenge when unusual histological features are not present and t(x;18) is negative. Clinically and radiologically, these tumors showed features identical to typical pulmonary synovial sarcomas, as well as histologically, with dense cellularity, interconnected fascicles, hyalinized stroma, hemangiopericytoma-like vascularization, focal myxoid changes, and encapsulated benign pulmonary epithelium.
The differentiation was made thanks to immunohistochemistry which showed the presence of the rare neoplasm compared to other pulmonary neoplasms A comparative study of primary pulmonary synovial neoplasm was carried out between a French case and the case detected by us confirmed at the Marius Nasta Institute in Bucharest

KEYWORDS
pulmonary synovial neoplasm, immunohistochemistry, tomography

Primary pulmonary synovial sarcoma is rare and presents a diagnostic challenge when unusual histologic features are not present Clinically and radiologically, these tumors presented features identical to typical pulmonary synovial sarcomas, as well as histologically, with dense cellularity, interconnected fascicles, hyalinized stroma, hemangiopericytoma-like vascularization, focal myxoid changes, and encapsulated benign pulmonary epithelium. and t(x;18) is negative.
They differ in the focal presence of unusual histological features not usually seen in pulmonary synovial sarcoma but typical of other neoplasms. These include Verocay bodies, rosettes, papillary structures with fibrovascular nuclei, adenomatoid changes, and rhabdoid morphology. Immunohistochemistry demonstrated typical expression of focal cytokeratins, vimentin, and occasionally CD99, Bcl-2, and smooth muscle actin.
These tumors were often negative or showed weak amplification for t(x;18). In conclusion, awareness of unusual focal histology in otherwise typical pulmonary synovial sarcoma may prevent misdiagnosis, especially when t(x;18) is negative.

Study carried by French researchers
Sixty known cases of pulmonary synovial sarcoma from 1981 to 2006 were retrieved from tissue archives. Seventeen cases containing unusual focal histological features were included. Tumors were subtyped as monophasic or biphasic according to World Health Organization criteria [7]. Grading according to tumor cell differentiation, mitotic rate, and necrosis was performed according to the French Federation of Cancer Centers (FNCLCC) scheme. Unusual histological features were noted and immunohistochemistry was performed on paraffin-embedded sections using commercially available antibodies.
They differ in the focal presence of unusual histological features not usually seen in pulmonary synovial sarcoma

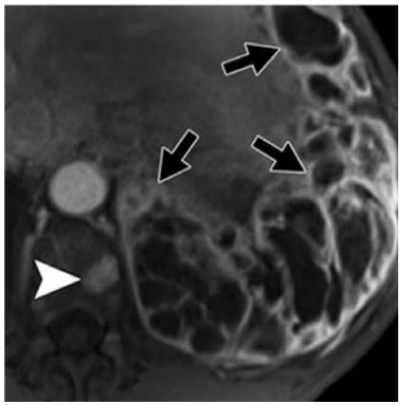
but typical of other neoplasms. These include Verocay bodies, rosettes, papillary structures with fibrovascular nuclei, adenomatoid changes, and rhabdoid morphology. Immunohistochemistry demonstrated typical expression of focal cytokeratins, vimentin, and occasionally CD99, Bcl-2, and smooth muscle actin.
These tumors were often negative or showed weak amplification for t(x;18). In conclusion, awareness of unusual focal histology in otherwise typical pulmonary synovial sarcoma may prevent misdiagnosis, especially when t(x;18) is negative. SYT/SSX RNA fusion transcripts resulting from the t(x;18) (p11;q11) translocation were detected using real-time reverse transcriptase polymerase chain reaction. Subtyping of SYT/SSX 1 and 2 fusion transcripts was performed using previously described methods.

	Clone	Titer	Source
Pancytokeratin	AE1/AE3	1:200	Roche, Mannheim, Germany
Cytokeratin-7	OV TL12/30	1:160	Dako, Carpinteria, CA
Epithelial membrane antigen	E29	1:100	Dako, Carpinteria, CA
Thyroid Transcription Factor-1	8G7G3/1	1:25	Dako, Carpinteria, CA
Cytokeratin 5/6	D5/16B4	1:20	Dako, Carpinteria, CA
Calretinin	CAL 3F5	1:50	Zymed, San Francisco, CA
Bcl-2	124	1:20	Dako, Carpinteria, CA
CD56	123C3	1:100	Caltag, Burlingame, CA
CD99	12E7	1:80	Dako, Carpinteria, CA
S-100	Polyclonal	1:800	Dako, Carpinteria, CA
Smooth Muscle Actin	1A4	1:800	Sigma, St. Louis, MO

Antibodies
Clinical features are not relevant for synovial neoplasm The study group included 6 men and 11 women aged 10 to 76 years (mean, 45). The most common presenting symptoms were dyspnea and shortness of breath. The tumors were distributed in the lung [8], pleura [8], and mediastinum [1]. Surgical procedures for the primary tumor included lobectomy [4], excision [8], and biopsy [5]. Local recurrence, metastasis, and survival data did not differ from those for pulmonary synovial sarcoma.

Mean age, years	**45**
Gender: Male Female	 6 11
Common symptoms: Dyspnea Chest pain Cough Pleural effusion	 5 5 4 3
Tumor location: Lung Pleura Mediastinum	 8 8 1
Treatment: Lobectomy Excision of mass Open biopsy	 4 8 5

Clinical Findings



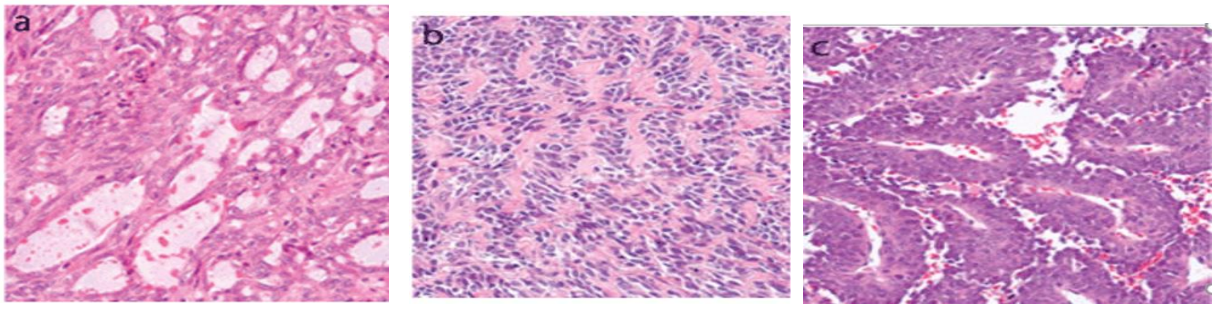
1.Synovial sarcoma in a 36-year-old man with dyspnea and pleuritic chest pain. Axial T2-weighted magnetic resonance image (2110/57.6) shows increased contrast between internal components, with well-demarcated spaces (arrowheads) suggesting cysts.

Mean tumor size, cm	7.5
Subtypes Monophasic Biphasic	 13 4
Unusual (common to other neoplasms, focally present) Verocay body-like areas Vague rosette formation Papillary structures Adenomatoid areas Rhabdoid morphology	 7 6 3 3 2

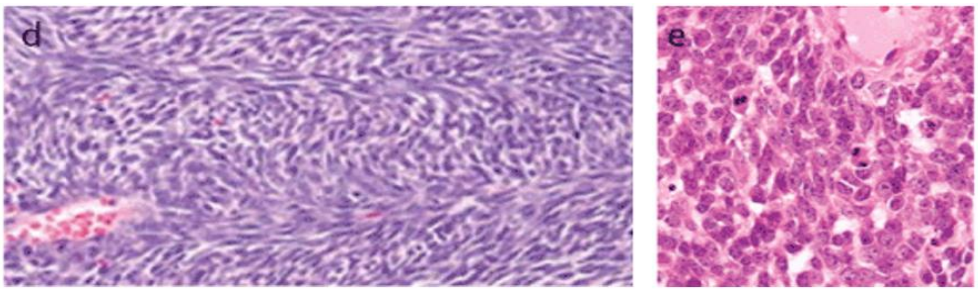
2.Gross and histologic findigns

Macroscopic and histological findings. The tumors ranged in size from 0.6 to 16 centimeters (mean, 7.5) and were well-circumscribed, soft, brownish masses with foci of necrosis, hemorrhage, and cystic changes. Histologically, the tumors were monophasic [10] or biphasic [4]. The tumors were grade 2 [11] and grade 3 (poorly differentiated, 6) according to the classification of the French Federation of Cancer Centers (FNCLCC)

Histologic features typical of pulmonary synovial sarcoma included dense cellularity, interconnected fascicles, and hyalinized or eosinophilic stroma. Hemangiopericytoma-like vascularization (15), focal myxoid changes [11], and benign encapsulated pneumocytes [12] were also observed. Unusual histologic features were focal, seen in at least 1 but no more than 4 slides per case, ranging from 4 to 90 high-power fields, and included Verocay body-like areas [7], vague rosette formation [6], well-formed papillary structures [3], adenomatoid areas [3], and rhabdoid morphology.. Typical histological features of pulmonary synovial sarcoma were observed, including dense cellularity, interconnected fascicles, and hyalinized or eosinophilic stroma. Hemangiopericytoma-like vascularization (15), focal myxoid changes [11], and encapsulated benign pneumocytes were also observed. Unusual histological features were focal, observed in at least 1 but no more than 4 slides per case, ranging from 4 to 90 high-power fields, and included Verocay body-like areas [7], vague rosette formation [6], well-formed papillary structures [3], adenomatoid areas [3], and rabd morphology.



3.Typical histologic features of pulmonary synovial sarcoma, characteristically composed of densely cellular interconnected fascicles (a). Unusual features that may lead to misdiagnosis include papillary formations (b), Verocay body-like areas (c), adenomatoid appearance



4.Histological features adenomatoid appearance (d) and rhabdoid morphology (e) or vague rosette formation.

Immunohistochemical and molecular findings

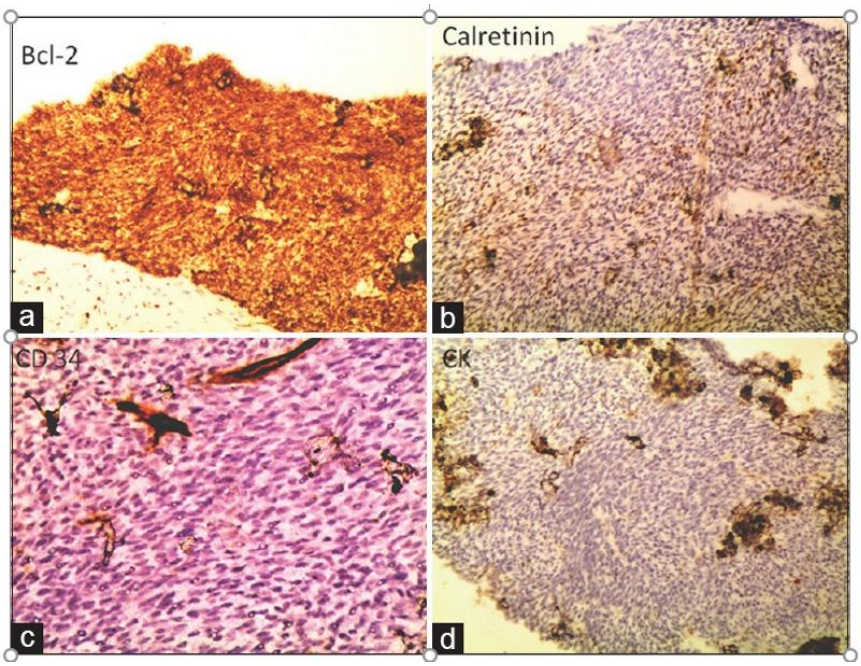
Immunohistochemical studies showed focal positive membranous or cytoplasmic staining for epithelial markers, including pancytokeratin (14), epithelial membrane antigen [8], and cytokeratin 7 [7], while three cases had focal immunoreactivity for all 3 epithelial markers. One tumor was also focally positive for cytokeratin 5/6. Diffuse immunoreactivity was seen with Bcl-2 in four cases and CD99 in seven cases. Focal immunoreactivity was present for CD56 [5], S-100 [4], calretinin [3], and smooth muscle actin [2]. Benign entrapped pneumocytes, present in seven cases, were immunoreactive for thyroid transcription factor-1 and epithelial markers.

Pancytokeratin	14
Epithelial Membrane Antigen	8
Cytokeratin -7	7
Calretinin	3
Cytokeratin 5/6	1
CD99	7
Bcl-2	4
CD56	5
S-100	4
Smooth Muscle Actin	2

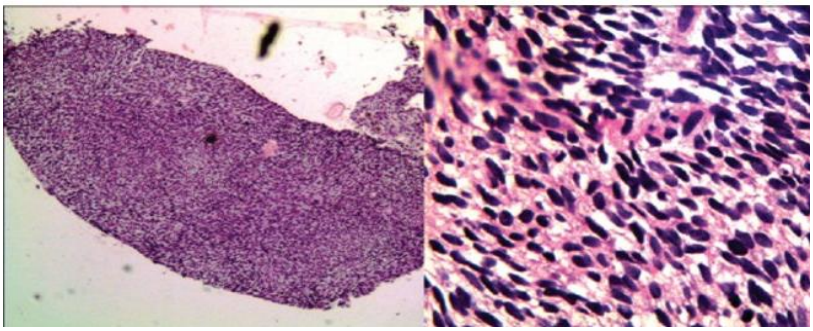
5.Imunohistochemical findings

Total t(x;18) positive	9
Total SYT/SSX1	3
Total SYT/SSX2	5
Total t(x;18) positive, SSX unknown	1
Total t(x;18) negative	8

6.Molecular findings



7.Photomicrograph of immunohistochemistry of computed tomography-guided tru-cut biopsy tissue, showing (a) bcl-2 positivity, (b) calretinin negativity, (c) CD34 negativity, and (d) cytokeratin negativity of spindle tumor cells.

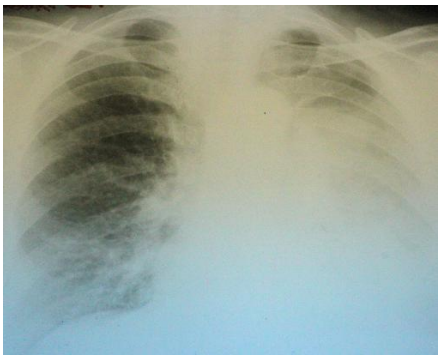


9.Photomicrograph of histopathology of CT-guided tru-cut biopsy of a right-sided lung mass showing malignant sarcomatoid neoplasm (H and E, ×10 and ×40)

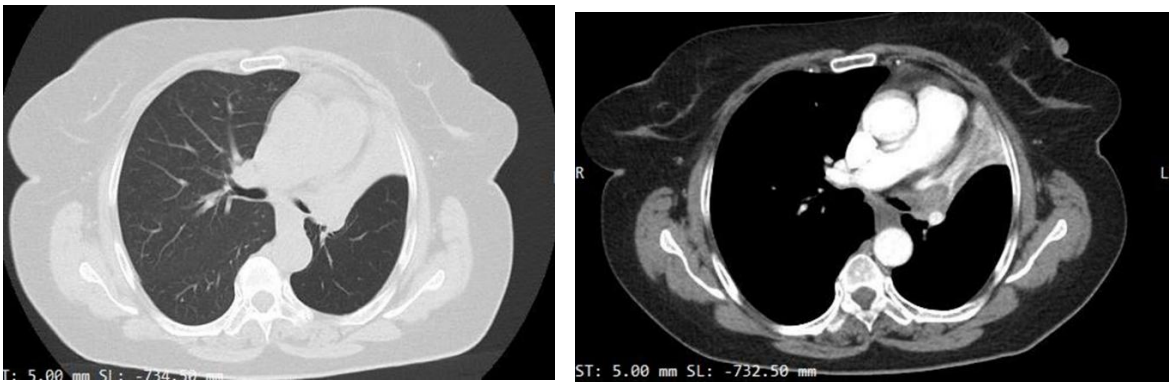
The case of synovial neoplasm studied

Patient S.M. presents to the Caracal Pulmonology Clinic with the following symptoms: Loss of appetite with moderate weight loss, pronounced dyspnea on low and medium exertion, asthenia, sweating, sometimes dry cough stab wound to the left hemithorax.

The objective examination reveals a blocked upper left hemithorax, globally diminished respiratory amplitude, and discrete bronchial rales in the left hemithorax. The patient undergoes a chest X-ray.

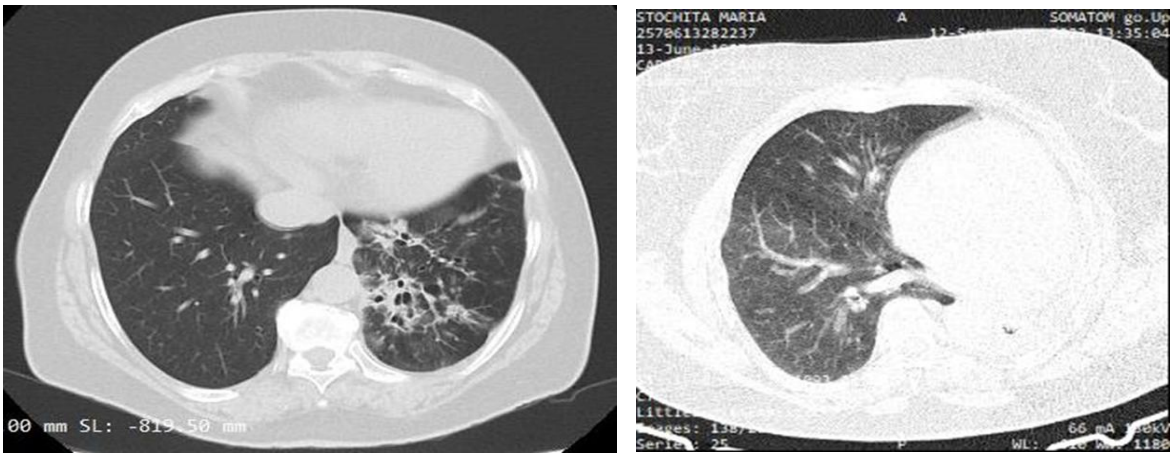


9.The lung X-ray performed on 06/16/2021 highlights an opacity of inhomogeneous costal intensity that encompasses almost 1/3 of the left hemithorax with poorly demarcated edges located at the level of the left pulmonary hilum. Based on clinical data corroborated with the pulmonary radiological image, it is decided to perform a computed tomography scan performed on 06/24/2021



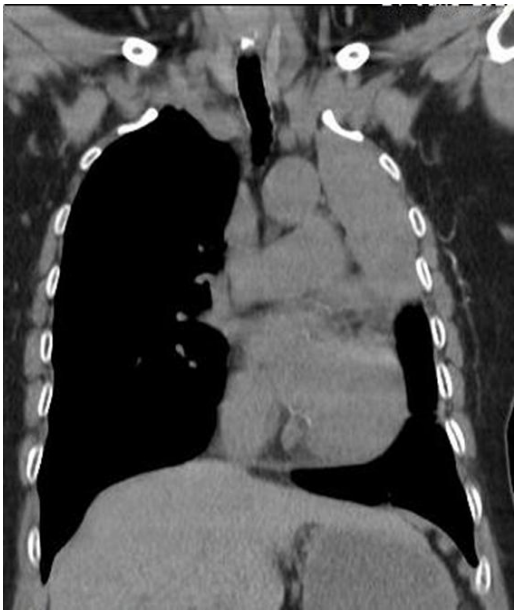
A B

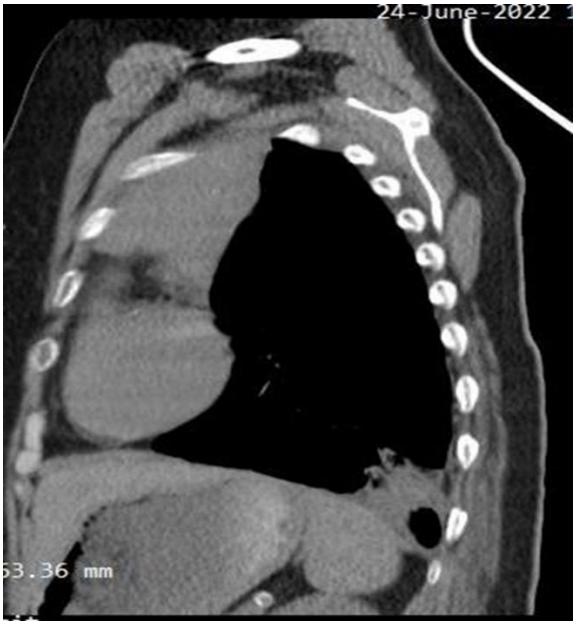
10.The 2 tomographic images A and B highlight the presence of the tumor in the left upper lobe B. performed with contrast substance



C D

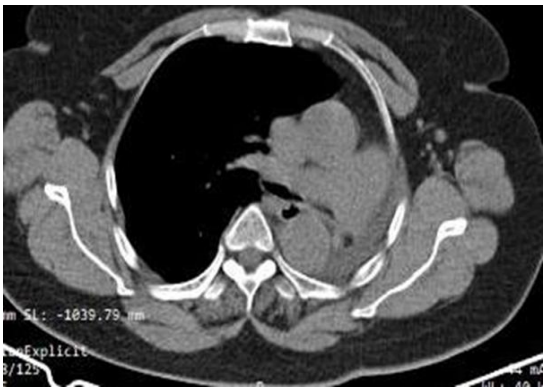
11.C highlights extensive bronchiectasis in the left lower lobe and CT D images are sequelae of an image that resembles Covid 19

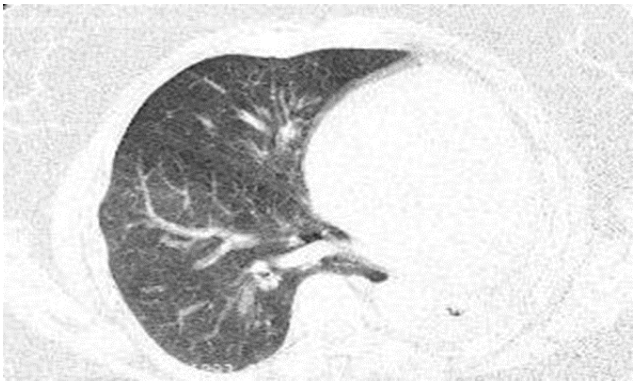




E H

12.Scans E and F show the position of the tumor in the posterior left upper lobe.
He was admitted in July to the Marius Nasta Institute of Pneumology in Bucharest where he underwent 3 bronchoscopies, the result of which was; from the first bronchoscopy;; larynx with dynamics present. Large tumor formation at the level of the left spur - obstructive aspect (from the level of the fourth bronchial cartilage).
Surgical intervention is performed with removal of the left superior lobe.
PATHOLOGICAL ANATOMY performed at the Marius Nasta Institute Tissue tumor fragments, consisting of a fasciculata sarcomatoid proliferation, of monotonous spindle cells, with mitoses; necrotic detritus
Multi-cytokeratin antibodies (AE1-AE3) negative; KI67 nuclear positive, with a mitotic index of approximately 70%; Sox 10 negative: STAT 6 negative: Tle 1 with intense, homogeneous positive nuclear labeling.
Conclusion; histopathological aspects and IHC tests argue for the diagnosis of Synovial Sarcoma, NOS, invasive bronchial ICD-O Code; 9040-3.
The patient undergoes immunological treatment and chemotherapy in Bucharest and presents in 2023 when a new computed tomography scan is performed.

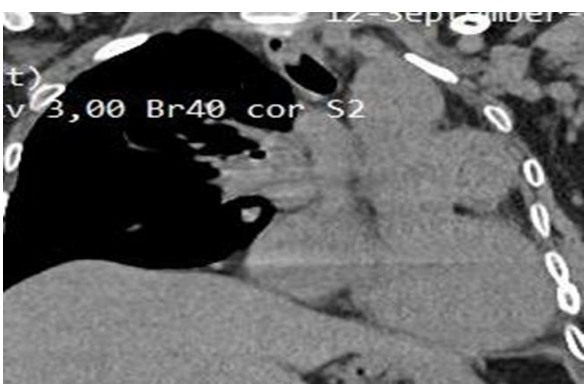


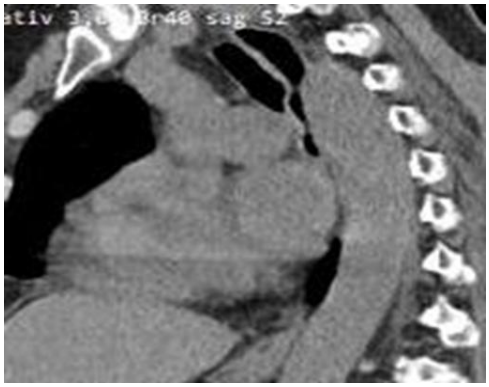


A B

13. The patient undergoes another control chest tomography in 2023 and A shows the mediastinal shift to the left and the presence of a tumor formation flanking the left mediastinum.In image B, the same components as in A are

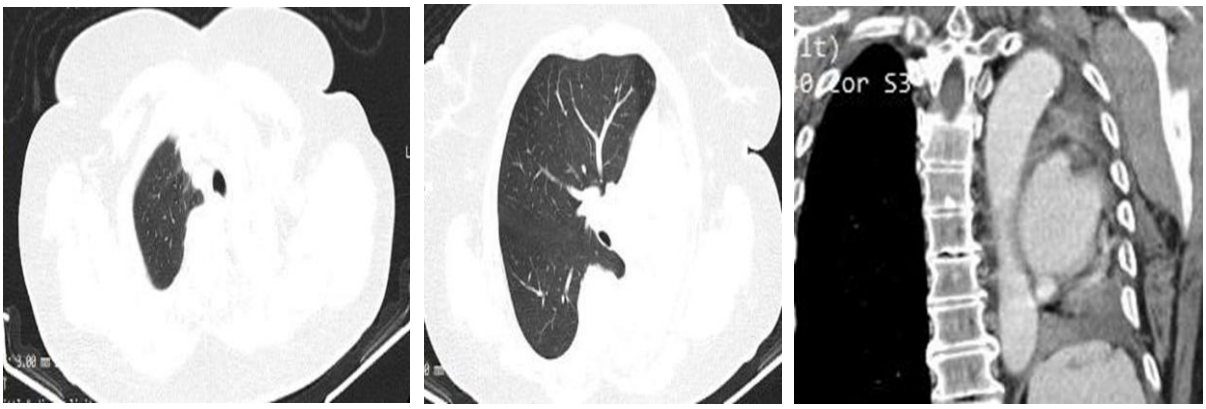
highlighted but it shows a ground-glass image in the right hemithorax - it should be noted that the patient contracted Covid-19 immediately after being transferred from the intensive care unit to the ward within the Marius Nasta Institute of Pulmonology.





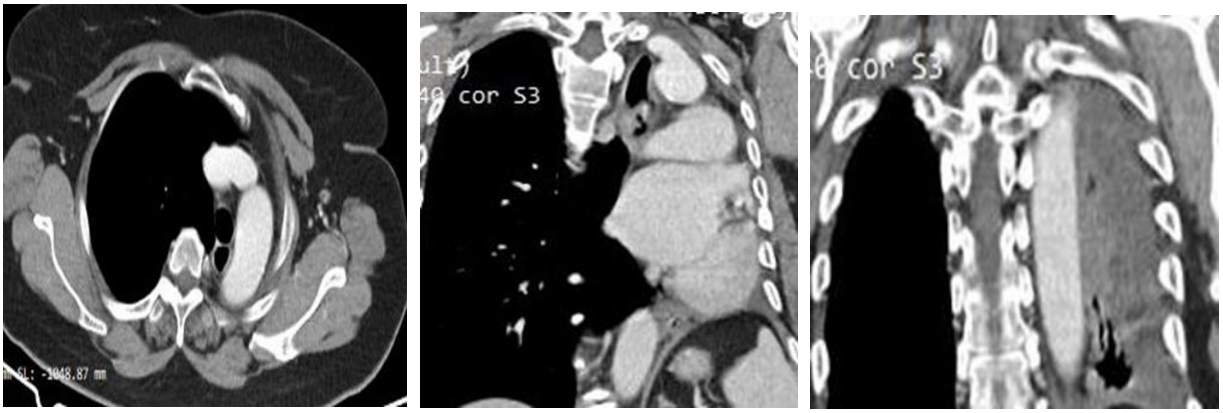
C D

14.CT scans C and D also illustrate the presence of the tumor in the left hemithorax region flanking the posterior wall.



A B C

15. 11.08.2025 performs a control chest tomography and the A.B.C images highlight the presence of the tumor with secondary atelectasis as well as the fibrosis formed at this level with the displacement of the mediastinum to the left and the ascent of the right lung into the thoracic cavity



C D E

16. C shows the aortic arch displaced far to the left
D the heart pushed far to the left due to pulmonary atelectasis of the left lower lobe
E. the descending aorta pushed by pulmonary fibrosis
All these images C,D,E, were enhanced with contrast material

Conclusions

1. We detected a synovial neoplasm confirmed by immunohistochemistry Marius Nasta Institute of Pneumology

2. The rarity of synovial neoplasms led us to present this rare case. It is possible that another one was present but immunohistochemical confirmation was not performed.

3. The patient is currently undergoing drug treatment for COPD with steroidal anti-inflammatories and beta-2 antagonist bronchodilators by daily puffs She is not undergoing any oncological treatment

4. The only suspicion of confirming the diagnosis of synovial pulmonary neoplasm is that the life span of the patients studied by the French researchers was very short.

References

1.Begueret H, Galateau-Salle F, Guillou L, Chetaille B, Brambilla E, et al. (2005) Primary intrathoracic synovial sarcoma: a clinicopathologic study of 40 t(x;18)-positive cases from the French Sarcoma Group and the Mesopath Group. Am J Surg Pathol 29: 339-346. [Crossref
]2. Bijwaard KE, Fetsch JF, Przygodzki R, Taubenberger JK, Lichy JH (2002) Detection of SYT-SSX fusion transcripts in archival synovial sarcomas by real-time reverse transcriptase-polymerase chain reaction. J Mol Diagn 4: 59-64. [Crossref]
3.Essary LR, Vargas SO, Fletcher CD (2002) Primary pleuropulmonary synovial sarcoma: reappraisal of a recently described anatomic subset. Cancer 94: 459-469. [Crossref]
4.Fletcher CD, Unni KK, Mertens FE (2002) World Health Organization Classification of Tumors. Pathology and Genetics of Tumors of Soft Tissue and Bone. IARC Press: Lyon, France pp. 427.
5..Hartel PH, Fanburg-Smith JC, Frazier AA, Galvin JR, Lichy JH, et al. (2007) Primary pulmonary and mediastinal synovial sarcoma: a clinicopathologic study of 60 cases and comparison with five prior series. Mod Pathol 20: 760-769. [Crossref]
6.Kleihues P, Cavenee WKE (2000) World Health Organization Classification of Tumors. Pathology and Genetics of Tumors of the Central Nervous System pp.172-17
7.Machen SK, Fisher C, Gautam RS, Tubbs RR, Goldblum JR (1998) Utility of cytokeratin subsets for distinguishing poorly differentiated synovial sarcoma from peripheral primitive neuroectodermal tumour. Histopathology 33: 501-507. [Crossref]
8.Miettinen ME (2003) Diagnostic Soft Tissue Pathology. Churchill Livingstone: New York pp. 463-468.
9.Okamoto S, Hisaoka 2021 Copyright OAT. All rights reserv al. (2004) Primary pulmonary synovial sarcoma: a clinicopathologic, immunohistochemical, and molecular study of 11 cases. Hum Pathol 35: 850-856. [Crossref]
10.Suster S, Moran CA (2005) Primary synovial sarcomas of the mediastinum: a clinicopathologic, immunohistochemical, and ultrastructural study of 15 cases. Am J Surg Pathol 29: 569-578. [Crossref]
11Smith TA, Machen SK, Fisher C, Goldblum JR (1999) Usefulness of cytokeratin subsets for distinguishing monophasic synovial sarcoma from malignant peripheral nerve sheath tumor. Am J Clin Pathol 112: 641-648. [Crossref]
12.Travis WD, Brambilla E, Muller-Hermelink HK, Harris CC (2004) World Health Organization Classification of Tumors. Pathology and Genetics of Tumors of the Lung, Pleura, Thymus, and Heart. IARC Press: Lyon, France pp. 344.
13.Zeren H, Moran CA, Suster S, Fishback NF, Koss MN (1995) Primary pulmonary sarcomas with features of

monophasic synovial sarcoma: a clinicopathological, immunohistochemical, and ultrastructural study of 25 cases. Hum Pathol 26: 474-480. [Crossref]

INSULIN RESISTANCE AND RIGHT VENTRICULAR DYSFUNCTION: PATHOPHYSIOLOGICAL MECHANISMS AND IMPLICATIONS FOR PULMONARY FUNCTION – A CRITICAL SYNTHESIS OF RECENT EVIDENCE

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DOI: https://doi.org/10.66793/titum19proceeding9

Abstract

Insulin resistance (IR) is a central component of the cardiometabolic syndrome, with major consequences on energy metabolism, endothelial function, and the cardio-respiratory system. Recent data show that IR does not affect only the left ventricle but also impacts the right ventricle (RV), which plays a pivotal role in maintaining pulmonary blood flow. Endothelial dysfunction associated with IR marked by reduced nitric oxide synthesis and activation of pro-inflammatory MAPK pathways contributes to pulmonary vascular stiffening and decreased myocardial compliance. These alterations lead to reduced TAPSE and S' values, as well as diminished alveolo-capillary diffusion capacity (DLCO) and forced expiratory volume (FEV_1), often before clinical symptoms appear.

Studies published between 2021 and 2025 have demonstrated an inverse relationship between HOMA-IR and RV performance, DLCO, and FEV_1, highlighting a pathophysiological link between insulin metabolism and cardio-respiratory function. Modern therapeutic agents, particularly SGLT-2 inhibitors, have been associated with improved endothelial function and reduced oxidative stress.

In conclusion, insulin resistance significantly contributes to early cardiopulmonary impairment in individuals with type 2 diabetes mellitus. Identifying these changes in their subclinical stages could enable more effective prevention and long-term preservation of cardiac and respiratory function.

I. Introduction

Type 2 diabetes mellitus represents one of the major challenges of modern medicine, through its multisystemic complications and its direct impact on cardiovascular and respiratory morbidity [1,2]. At the core of this metabolic syndrome lies insulin resistance a silent biochemical disorder that profoundly alters cellular energy metabolism, chronic inflammation, and endothelial function [3,4].

While historically IR was regarded mainly through the lens of glycemic control, recent research has reframed it as a systemic dysfunction, redefining diabetic cardiomyopathy as part of a broader cardiometabolic disorder [5,6].

Emerging studies suggest that IR affects not only the left ventricle but also the right ventricle (RV) a cardiac chamber often overlooked in clinical assessments, yet essential for maintaining pulmonary hemodynamics [7,8]. IR promotes progressive vascular resistance and reduced ventricular compliance by altering microcirculation and pulmonary vascular tone [9,10]. Clinically, these mechanisms manifest as early right ventricular dysfunction detectable on imaging even before symptoms arise [11].

The effects of this cascade extend beyond the heart, influencing the lungs and respiratory mechanics. Given the close interdependence between the RV and pulmonary circulation, right ventricular dysfunction directly impairs lung mechanics and volumes, leading to reduced forced vital capacity (FVC), FEV_1, and decreased gas exchange efficiency [12,13].

This interaction between glucose metabolism, the heart, and the lungs suggests the existence of a functional insulin resistance right ventricle lung axis, potentially useful for identifying early subclinical complications in diabetic patients [14].

Within this context, the present paper provides a critical synthesis of current international evidence, focusing on the pathophysiological mechanisms linking insulin resistance to RV dysfunction and reduced pulmonary volumes. The dual aim is: (1) to clarify the molecular and clinical basis of this association, and (2) to integrate recent translational findings into a preventive and patient centered framework for the management of diabetes.

II. Pathophysiological and Molecular Mechanisms of the Insulin Resistance–Right Ventricle–Lung Interaction

II.1. The Endothelium: The Fragile Link in Insulin Resistance

In IR, the earliest manifestation is not metabolic but vascular. The endothelium once a dynamic regulator of vascular tone and inflammation becomes a driver of stiffness and diffuse microinflammation.

Reduced activity of the PI3K–Akt pathway and concurrent activation of MAPK–ERK disrupt the balance between vasodilation and vasoconstriction, while decreased nitric oxide synthesis leads to altered pulmonary microcirculatory flow.

In patients with type 2 diabetes, these mechanisms translate into subtle but consistent reductions in respiratory volumes (FVC, FEV_1), even among individuals without diagnosed lung disease [15].

II.2. The Right Ventricle – The Subtle Metabolic Component

The right ventricle responds quietly but sensitively to metabolic stress. Under IR conditions, cardiomyocytes lose their ability to oxidize fatty acids efficiently, producing less ATP and more reactive oxygen species.

This mitochondrial dysfunction creates a vulnerable microenvironment characterized by interstitial fibrosis, decreased compliance, and early reduction in TAPSE and RV strain echocardiographic markers that may precede clinical symptoms [11].

Recent studies indicate that the reduced adaptive capacity of the right ventricular myocyte explains its heightened sensitivity to insulin resistance [16,17].

Conversely, modern multitarget therapies such as SGLT-2 inhibitors seem to provide real benefits by lowering oxidative stress and systemic inflammation, as shown by Pârliteanu and Nemeș (2025) [18]. Their antioxidant and endothelium-protective effects open promising translational avenues for restoring cardio-pulmonary balance.

II.3. The IR–RV–Lung Axis: A Functional Interaction

As the right ventricle stiffens and diastolic pressure rises, this pressure is transmitted retrogradely into the pulmonary circulation. The result is reduced alveolar compliance, with spirometry showing a consistent, mild decline in FVC and DLCO correlated with the degree of IR and endothelial dysfunction [19,20].

In this view, IR cannot be considered in isolation; it binds metabolism, the heart, and the lungs in a common pathological circuit where hypoxia worsens IR, and IR exacerbates hypoxia.

Such an integrative perspective supports early intervention based on coordinated assessment of metabolic, endothelial, and right ventricular function aimed at preventing cardiopulmonary deterioration in diabetic patients.

II.4. Functional Correlations Among Cardiac, Metabolic, and Pulmonary Parameters (TAPSE, HOMA-IR, DLCO, FEV_1)

Clinically, the expression of insulin resistance induced cardiometabolic dysfunction often manifests through subtle changes detectable only by correlating cardiac and respiratory parameters.

At the cardiac level, one of the earliest detectable alterations is a mild reduction in TAPSE (Tricuspid Annular Plane Systolic Excursion), a sensitive marker of longitudinal RV contractility. Decreases in S' and right ventricular strain similarly indicate early impairment of myocardial performance, preceding overt heart failure symptoms.

These changes frequently correlate with elevated HOMA-IR, reflecting systemic insulin resistance and cellular metabolic imbalance [19].

At the respiratory level, mild reductions in DLCO (alveolo-capillary diffusion capacity), FEV_1, and FVC are often observed indicating early impairment in gas exchange and reduced pulmonary compliance, commonly linked to venous congestion secondary to RV diastolic dysfunction [20].

Overall, the relationship among metabolic (HOMA-IR), cardiac (TAPSE, S'), and respiratory (DLCO, FEV_1) parameters suggests a unified functional axis through which insulin resistance directly modulates cardio-pulmonary capacity.

III. Statistical Synthesis

III.1. Right Ventricular Dysfunction – The Subclinical Expression of Insulin Resistance

Recent evidence confirms that IR directly impacts RV performance even before overt clinical manifestations.

The European multicenter study led by Martínez-Sellés et al. (2023), including over 1,200 patients with type 2 diabetes, reported significant reductions in TAPSE (−1.9 mm; $p < 0.001$) and S' (−0.7 cm/s; $p < 0.01$) in subjects with HOMA-IR > 3 [21].

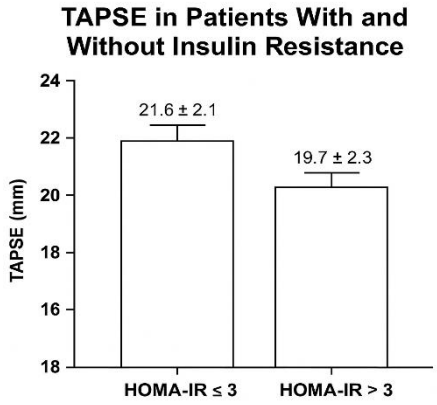

Figure 1. Comparison of right ventricular systolic function (TAPSE) in patients with and without insulin resistance.

Patients with insulin resistance (HOMA-IR > 3) exhibited a significantly lower mean TAPSE value (19.7 ± 2.3 mm) compared with those with preserved insulin sensitivity (HOMA-IR ≤ 3; 21.6 ± 2.1 mm), suggesting an early subclinical impairment of right ventricular longitudinal contractility associated with metabolic dysfunction.

Moreover, right ventricular longitudinal strain varied inversely with HOMA-IR ($r = −0.42$, $p < 0.01$), suggesting that metabolic myocardial dysfunction precedes structural remodeling.

These findings indicate that the right ventricle may serve as an early functional marker of systemic metabolic stress, even when the left ventricle remains unaffected.

III.2. Early Respiratory Impairment – An Indirect Marker of Cardiometabolic Dysfunction From a respiratory standpoint, recent literature supports that insulin resistance affects pulmonary function independently of obesity or smoking.

The meta-analysis by Lee et al. (2024), including 8,317 participants from 15 cohorts, demonstrated a mean reduction of 6.4% in FEV_1 and 7.2% in DLCO among individuals with severe IR compared with insulin-sensitive controls [22].

The negative correlation between DLCO and HOMA-IR remained significant after adjustment for age, BMI, and inflammatory status.

The authors emphasized that decreased alveolo-capillary diffusion capacity is not merely a consequence of pulmonary congestion but reflects diffuse microvascular inflammation driven by systemic endothelial dysfunction induced by IR.

III.3. The Cardio-Pulmonary Correlation – An Integrated Phenotype of Insulin Resistance

The global DYNAMIC study (Gupta et al., 2025), conducted on 6,700 patients with type 2 diabetes, further consolidated the concept of a cardio-pulmonary phenotype of insulin resistance [23].

Study	Year	Population (n)	Main Parameters	Key Finding	p-value
Martínez-Sellés et al.	2023	1,200 T2DM	TAPSE, S', HOMA-IR	Significant inverse correlation between insulin resistance and TAPSE (r = −0.42)	<0.01
Lee et al.	2024	8,317 (15 cohorts)	FEV_1, DLCO	6.4% decrease in FEV_1 and 7.2% decrease in DLCO in severe insulin resistance	<0.001
Gupta et al. (DYNAMIC)	2025	6,700 T2DM	TAPSE, DLCO, FEV_1	Concurrent decline in TAPSE, DLCO, and FEV_1 with increasing insulin resistance	<0.01

Table 1. Summary of the main studies

In this population, rising HOMA-IR was associated with a simultaneous decrease in TAPSE (−1.5 mm), DLCO (−5.9%), and FEV_1 (−4.8%), with a 2.3-fold higher risk of restrictive ventilatory dysfunction ($p < 0.01$).

In summary, insulin resistance contributes to early right ventricular and pulmonary dysfunction through endothelial and metabolic pathways. Recognizing this integrated cardiometabolic–respiratory axis may enable earlier prevention and improved long-term outcomes in type 2 diabetes.

References

1. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nat Rev Endocrinol. 2018;14(2):88–98.

2. Cho NH, et al. IDF Diabetes Atlas, 10th ed. Diabetes Res Clin Pract. 2021;183:109119.

3. Samuel VT, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. Cell. 2012;148(5):852–871.

4. Petersen MC, Shulman GI. Mechanisms of insulin action and insulin resistance. Physiol Rev. 2018;98(4):2133–2223.

5. Jia G, Hill MA, Sowers JR. Diabetic cardiomyopathy: An update of mechanisms contributing to this clinical entity. Circ Res. 2018;122(4):624–638.

6. Bugger H, Abel ED. Molecular mechanisms of diabetic cardiomyopathy. Diabetologia. 2014;57:660–671.

7. Vonk Noordegraaf A, et al. The right ventricle revisited: physiology and pathophysiology in health and disease. Eur Heart J. 2019;40(1):17–24.

8. Alpert MA, Karthikeyan K, Abdullah O, Ghadban R. Diabetic cardiomyopathy: Mechanisms and therapeutic implications. Am J Med Sci. 2018;355(4):266–273.

9. De Boer RA, et al. Diabetic cardiomyopathy in type 2 diabetes: current insights and future directions. Front Physiol. 2023;14:1124561.

10. Mele D, et al. Right ventricular dysfunction in diabetes mellitus: an emerging issue. J Am Coll Cardiol. 2020;75(7):1019–1030.

11. Di Bello V, et al. Insulin resistance and early right ventricular dysfunction: echocardiographic assessment. Eur Heart J. 2021;42(19):1901–1913.

12. Frøbert O, et al. Lung function and insulin resistance: a population-based study. Diabetes Care. 2022;45(5):1150–1158.

13. Crippa M, et al. Insulin resistance and pulmonary function: the metabolic–respiratory connection. Front Physiol. 2023;14:1194522.

14. Radu D, et al. The impact of insulin resistance on lung volume through right ventricular dysfunction in diabetic patients—literature review. J Pers Med. 2023;15(8):336.

Referințe (secțiunea II, de la nr. 15)

15. Nemeș RM, et al. Early respiratory impairment in type 2 diabetes. Romanian J Intern Med. 2022;60(4):298–306.

16. Bugger H, Abel ED. Mitochondrial dysfunction in insulin resistance and diabetic cardiomyopathy. Nat Rev Endocrinol. 2023;19(2):112–126.

17. Finck BN, Kelly DP. PGC-1 coactivators and metabolic adaptation in the diabetic heart. Front Physiol. 2023;14:1108943.

18. Pârliteanu O-A, Nemeș R-M, Balteanu MA, Radu D, Gherlan G. Pathophysiological mechanisms and benefits of SGLT-2 inhibitors in a patient with cerebral artery aneurysm: A case report. Exp Ther Med. 2025;29(6):112.

19. Aimo A, Vergaro G, Castiglione V, et al. Right ventricular function in metabolic disorders: emerging links and prognostic implications. Eur J Heart Fail. 2023;25(7):1192–1203.

20. Celli BR, Agustí A. Pulmonary dysfunction in metabolic syndrome and diabetes: clinical relevance and diagnostic insights. Lancet Respir Med. 2024;12(2):134–146.
Referințe (Secțiunea III)

21. Martínez-Sellés M, et al. Insulin resistance and subclinical right ventricular dysfunction in type 2 diabetes: a multicenter analysis. Eur Heart J Cardiovasc Imaging. 2023;24(6):872–881.

22. Lee SW, Kim YJ, Lim JH, et al. Insulin resistance and impaired lung function: a meta-analysis of population-based studies. Chest. 2024;166(2):345–358.

23. Gupta A, Prakash S, Chowdhury A, et al. Integrated cardiopulmonary consequences of insulin resistance: insights from the global DYNAMIC study. J Clin Endocrinol Metab. 2025;110(3):e458–e469.

3D PRINTING AND BIOPRINTING: TODAY AND TOMORROW IN THE ORTHOPEDIC FIELD – HORIZON 2030 - 2050

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DOI: https://doi.org/10.66793/titum19proceeding10

Abstract: *3D printing, or additive manufacturing, profoundly transforms the orthopedic field by allowing the design and production of custom implants from patient-specific digital data. Unlike CAD/CAM, which is based on subtractive machining, 3D printing relies on the addition of successive layers of material, opening the way to porous, light and biomimetic structures that promote osseointegration. Born in the 1980s with the SLS laser CO in Austin (Texas), this technology has extended to metals, ceramics and polymers. Today, bio-printing adds a biological dimension, integrating cells and matrices to manufacture tissues and living grafts. By 2030-2050, the convergence between engineering, artificial intelligence and biology will allow the production of personalized organs and living hybrid implants, marking the advent of printed and customized regenerative medicine.*

1. Introduction

For more than two decades, three-dimensional printing (3D printing) – also called additive manufacturing – has established itself as a disruptive technology in many industrial fields. In orthopedics, it is no longer limited to the production of prototypes: it redefines the design, manufacturing and customization of implants and medical devices.

From computer-aided design (CAD) to computer-aided manufacturing (CAM), to bioprinting of living tissues, the advances are dazzling. 3D printing marks, according to several observers, the beginning of a third industrial revolution, following the mass production of the 20th century. By 2030-2050, it should be fully integrated into surgical practice and regenerative medicine.

2. Clarify the difference between CAD/CAM and 3D Printing

In the medical field, and particularly in orthopedics, the terms CAD/CAM (Computer-Aided Design / Computer-Aided Manufacturing) and 3D Printing (Additive Manufacturing) are often used interchangeably, although they refer to two fundamentally different approaches. This confusion is frequent, as the two technologies share the same numerical basis – computer-aided design – but deeply diverge in their manufacturing principles.

The CAD/CAM system is based on a subtractive process: a part is machined from a raw block (metal, ceramic or polymer) by milling, turning or grinding. This is a high-precision process, perfectly suited to hard materials (CoCr, zirconia, titanium). On the other hand, it generates a significant loss of material, requires costly mechanical tooling and offers limited geometric flexibility (Fig.1).

3D printing, or additive manufacturing, proceeds in the opposite way: the part is built layer by layer from a powder, a filament or a resin (Fig.2). This mode of production allows complex geometries, porous internal structures and patient-specific customization impossible by conventional machining. On the other hand, it presents technical challenges: residual stresses, surface roughness, internal porosity and still incomplete validation of long-term mechanical properties **Table 1**

Table 1 Comparative Aspect CAD/CAM -3D Printing

Comparative aspect	CAD/CAM (subtractive)	3D Printing (additive)
Principle	Material removal (machining)	Successive layer deposition
Typical materials	Metal or ceramic blocks	Powders, polymers, metals, ceramic
Dimensional accuracy	Excellent (µm)	Good to medium depending on the process
Freedom of design	Very high	Limited design freedom (internal structures, porosity)
Material loss	High	Minimal
Production speed	Fast for standard series	Slower, suitable for single piece
Typical applications	Dental crowns, standard prostheses, milled implants	Custom implants, porous bone structures, biological prototypes

Thus, CAD/CAM remains essential for devices requiring high geometric precision and hard materials, while 3D printing opens the way to biological and structural customization of implants, particularly in regenerative orthopaedics.

3. 3D printing technologies and historical evolution

3D printing is based on the superposition of successive layers of material from a digital model (CAD file). The technologies according to the materials used and the clinical needs are:

- Selective laser sintering (SLS) and selective laser melting (SLM): mainly for metal alloys (titanium, cobalt-chromium, steels, aluminum).
- Direct metal laser sintering (DMLS)
- Electron beam fusion (EBM)
- Photopolymerization (SLA, DLP): suitable for anatomical models and surgical guides.

History, Origins: the school in Austin (Texas) and the CO laser

The modern history of 3D printing begins in the 1980s in the United States.

In 1986, Chuck Hull founded 3D Systems and invented stereolithography (SLA), the first patented additive manufacturing process.

Almost simultaneously, at the University of Texas at Austin, Dr. Carl Deckard and Professor Joe Beaman are developing Selective Laser Sintering (SLS), using a CO laser to selectively fuse polymer or metal powders.

This invention, marketed by their company Desk Top Manufacturing (DTM Corp.), laid the foundation for modern metal 3D printing before being integrated into 3D Systems in 2001.

This "school of Austin" has thus transformed the CO laser into an industrial production tool, opening the way to the manufacture of implants and custom medical devices.

Figure 1 CAD / CAM Techology

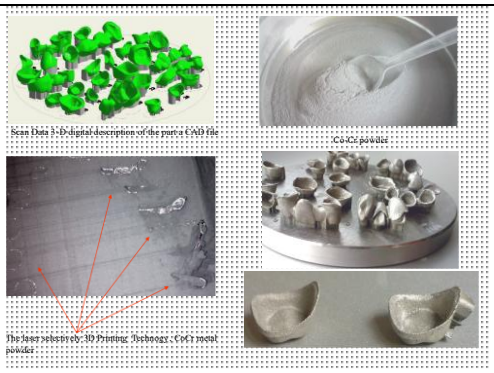

Figure 2 3D Printing Technology

4. 3D printing technologies applied to orthopaedics

Different technologies coexist according to the materials used and the precision requirements:

- Selective laser sintering (SLS) and selective laser melting (SLM): widely used for metal alloys (titanium, cobalt-chromium, stainless steel, aluminum).
- Direct metal sintering by laser (DMLS): process developed by the German company EOS GmbH, always active and world leader in metal and polymer 3D printing.
- Electron beam fusion (EBM): technology invented by ARCAM AB (Sweden), now part of the GE Additive group, used for medical titanium implants.
- Photopolymerization (SLA, DLP): suitable for producing anatomical models, surgical guides and prototypes.
- Extrusion (FDM, DIW): used for polymers, ceramics and composite materials.

Data from the scanner or medical imaging are transformed into CAD files, then printed layer by layer. This digital chain – scan, modeling, manufacturing – allows for complete customization, essential in modern orthopedic treatment (Fig. 3)

Figure 3 3D Printings in orthopedic treatment

5. Current medical applications

3D printing is today used to manufacture personalized implants and prostheses, perfectly adapted to the anatomy of each patient. The alloys Ti6Al4V, Ti-Nb-Zr and Co-Cr are preferred for their mechanical properties and biocompatibility.

The porous structures obtained by laser fusion promote osteointegration, improving stability without resorting to a bone cement.

- ***The Fraunhofer institutes (ILT, Germany***) are actively pursuing their research on laser processes for implants, in particular on bio-resorbable materials (calcium phosphate, polylactide).
- ***EOS GmbH (Munich)*** continues to be a pioneer in the development of printed vertebral and acetabular implants.
- ***In the United States, ConforMIS Inc***., specialized in custom knee prostheses, has demonstrated equivalent or even superior clinical performances to standard implants; since 2023, the company is part of the restor3d, Inc. group, which pursues these personalized developments.

6. From 3D printing to bio-printing

The next step is three-dimensional bioprinting, which aims to reproduce living biological tissues from human cells and biomaterials.

Three approaches structure this field:

- ***Biomimetics***, which seeks to faithfully reproduce natural cellular and extracellular architectures
- ***Cellular self-assembly***, inspired by the processes of embryonic development
- ***Functional mini-tissues***, modular elements used to reconstruct complete organs. Several industrial and academic actors contribute to this progress.

The companies

- ***L'Oréal and BASF***, in partnership with the start-up Poietis (France, now inactive since 2025), have developed human skin bioprinting processes for cosmetic and medical purposes
- ***The Wallenberg Wood Science Center (Sweden***) demonstrated the feasibility of printing ear cartilage from human cells and biopolymers derived from algae.
- ***Finally, the American company Organovo, now renamed VivoSim Labs Inc***., continues its work on liver and kidney tissues printed for in vitro pharmacological tests.

These advances signal the next step: the production of tissue grafts and, in the longer term, complete organs printed on demand.

7. Outlook to 2025–2030

In the short term (2025-2030), 3D printing will become a standard tool for surgical planning and production of individualized implants.

In the medium term (2030-2050), the convergence between materials engineering, artificial intelligence and bioprinting will pave the way for tailor-made regenerative medicine.

Classical metal implants could be replaced, in some indications, by bio-printed structures integrating stem cells and biodegradable matrices.

The rise of manufacturing on demand will transform the medical supply chain: decentralized production, short circuits and increased sustainability.

This evolution is fully in line with a third industrial revolution, combining digital technologies, biology and medicine.

8. Conclusion-2030

3D printing, and now bio-printing, are redefining the contours of modern orthopaedics.
From the printed metal prosthesis to the bio-fabricated organ, the progress made in less than twenty years is remarkable.
The challenges remain: mastery of micro-architecture, clinical validation, standardization of processes and long-term biological compatibility.
But the dynamic is irreversible: 3D printing is becoming a key pillar of personalized and regenerative medicine in the 21st century.
Horizon 2030: 3D printing will no longer be a technological curiosity, but an integrated ***therapeutic standard*** in surgery and tissue regeneration.

9. Bioprinting in 2050: towards complete regenerative medicine

By 2050, bioprinting should reach a level of technological and clinical maturity that will disrupt the foundations of restorative medicine.
Current advances – today still limited to simple tissues or research prototypes – will evolve towards functional therapeutic applications, based on four main axes:
a)- *Functional and vascularized bio-printed organs*
The joint progress of multicellular bioprinting, microfluidics and bio-inks will create vascularized organs, ensuring internal perfusion and oxygenation.
In 2050, it will be realistic to print partially functional livers, miniaturized kidneys, or even segmental hearts intended to replace defective organs.
These grafts will be printed from the patient's autologous cells, eliminating the risk of immunological rejection and reducing dependence on organ donation.
b- *Hybrid implants: materials + cells*
The integration of metal additive manufacturing and bioprinting processes will lead to a new generation of living hybrid implants.
An orthopedic implant made of titanium or a bio-resorbable alloy can, for example, be covered or infiltrated with osteoblastic cells from the patient, promoting natural and accelerated bone regeneration.
The boundaries between prosthesis and tissue graft will fade: the prosthesis will become an active biological support.
c- *An in situ and automated bioprinting*
The portable, sterile and robotic bioprinters will allow printing directly in the patient's bed or in the operating room.
The first prototypes of this type already exist for skin repair; in 2050, this approach could extend to bone, cartilage and muscle reconstruction after trauma.
The association between intelligent surgical robots and miniaturized bioprinters will allow fully personalized interventions, guided by real-time imaging.
d- *Integrated digital and ethical platforms*
Artificial intelligence and the patient's digital twins will play a central role.
Each printed organ will be preceded by a computer simulation of its morphology, mechanics and integration into the organism.

These virtual models will allow us to test the behavior of the plugin even before its printing.
In parallel, the ethical, regulatory and cell ownership stakes will become crucial: to whom belong the tissues printed from human cells? How to ensure biological safety?

In 2050, bioprinting will no longer be an experimental laboratory, but a major clinical pillar:
• the production of autologous replacement organs;
• personalized tissue repair;
• post-traumatic functional regeneration;
• the radical reduction of the need for classical transplantation.

It will symbolize the fusion of life and digital fabrication, uniting biology, engineering and computing, AI, and Learning Quantum Machine (LQM) in a same therapeutic approach.
Thus, the regenerative medicine of 2050 will probably be printed, connected and biological.

References

1. Hull, C. W. (1986). *Apparatus for production of three-dimensional objects by stereolithography.* U.S. Patent 4575330.
2. Première invention et brevet de la stéréolithographie (SLA), fondant la société 3D Systems.
Deckard, C. R. (1989). *Method and apparatus for producing parts by selective sintering.* U.S. Patent 4863538.
3. Invention du Selective Laser Sintering (SLS) à l'Université du Texas, Austin.
Beaman, J. J., Deckard, C. R., et al. (1992). *Selective Laser Sintering Additive Manufacturing Technology.* University of Texas at Austin.
4. Publication académique fondatrice sur le SLS et l'usage du laser CO_2.
Gibson, I., Rosen, D. W., & Stucker, B. (2015). *Additive Manufacturing Technologies: 3D Printing, Rapid Prototyping, and Direct Digital Manufacturing.* Springer, 2nd ed.
5. Murr, L. E. (2016). *Frontiers of 3D Printing/Additive Manufacturing in Biomaterials, Tissue Engineering, and Organ Printing. Journal of Materials Science & Technology*, 32(10), 987–995.
8.Synthèse des procédés métal et céramique appliqués aux implants médicaux.
9. Berman, B. (2012). *3-D printing: The new industrial revolution. Business Horizons*, 55(2), 155–162.
10. Discussion sur la personnalisation massive et la révolution industrielle associée.
11. Bose, S., Ke, D., Sahasrabudhe, H., & Bandyopadhyay, A. (2018). *Additive manufacturing of biomaterials. Progress in Materials Science*, 93, 45–111.
12.Mavrogenis, A. F., Igoumenou, V. G., et al. (2020). *3D printing of orthopedic implants: clinical experience and future perspectives. Expert Review of Medical Devices*, 17(7), 607–616.
13.Murphy, S. V., & Atala, A. (2014). *3D bioprinting of tissues and organs. Nature Biotechnology*, 32(8), 773–785.
14.Groll, J., Burdick, J. A., Cho, D.-W., et al. (2018). *A definition of bioinks and their distinction from biomaterials. Biofabrication*, 11(1), 013001.
15. Mandrycky, C., Wang, Z., Kim, K., & Kim, D.-H. (2016). *3D bioprinting for engineering complex tissues. Biotechnology Advances*, 34(4), 422–434.
16. Gatenholm, P., et al. (2016). *3D bioprinting of cartilage and soft tissues using nanocellulose bioinks. ACS Biomaterials Science & Engineering*, 2(10), 1705–1713.
17. Organovo Holdings Inc. (2024). *Corporate Research Summary: 3D Human Tissues for Drug Discovery and Regenerative Medicine.* [VivoSim Labs Inc., San Diego].
18.EOS GmbH – *Company Overview and Medical Applications.* (2024). https://www.eos.info/Fraunhofer Institute for Laser Technology ILT – *Additive Manufacturing and Bioprinting Projects.* (2024). https://www.ilt.fraunhofer.de/
19. GE Additive (ex-ARCAM AB) – *Electron Beam Melting Technology Overview.* (2024). https://www.ge.com/additive/
20. restor3d Inc. – *Acquisition of ConforMIS and custom orthopedic implant production.* (Press release, 2023).

THERAPEUTIC AND ANTICANCER INSIGHTS INTO TARAXACUM OFFICINALE BIOACTIVE COMPOUNDS

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DOI: https://doi.org/10.66793/titum19proceeding11

Abstract

Cancer remains one of the leading global health challenges, motivating continuous exploration for new therapeutic agents. Taraxacum officinale (dandelion) has been long used in traditional medicine for its wide range of pharmacological effects. Recent research has emphasized its potential anticancer properties, attributed to a diverse phytochemical composition including flavonoids, terpenoids, phenolic acids, and phytosterols. This review synthesizes current findings regarding the plant's ethnomedicinal uses, phytochemical profile, and anticancer mechanisms identified in in vitro and in vivo studies. Key compounds such as taraxasterol, chicoric acid, chlorogenic acid, and taraxinic acid demonstrate potential to inhibit cancer cell proliferation, induce apoptosis, and regulate oncogenic signaling pathways. Given its safety and multi-target activity, Taraxacum officinale represents a promising candidate for developing plant-derived anticancer therapeutics. Further preclinical and clinical studies are essential to confirm efficacy and clarify mechanisms of action.

Key words: taraxacum officnale, cancer, tarxainic acid , phytochemicals

Introduction

Cancer remains one of the leading causes of death worldwide, second only to cardiovascular diseases. It is characterized by the uncontrolled proliferation of malignant cells. According to recent estimates, cancer accounted for nearly 10 million deaths globally in 2020 [1]. In Romania, it represents the second most common cause of mortality after cardiovascular disease, responsible for about 19% of deaths, with over 95,000 new cases and nearly 54,000 fatalities reported annually [2].

In recent decades, research on plant-derived compounds has become essential in the search for novel anticancer agents. More than 35,000 plant species have been examined for potential oncological applications [3], and approximately 3,000 are recognized for their abundance of anticancer constituents. Notably, over 60% of anticancer drugs currently used are derived from natural sources, primarily from plants and animals [4]. Modern technologies, such as virtual screening, molecular modeling, and advanced analytical and bioassay techniques, have accelerated the identification of new natural therapeutic candidates [5,6].

Among medicinal plants, *Taraxacum officinale* H. Wigg. (common dandelion) has attracted increasing scientific attention for its rich phytochemical profile and promising antitumor potential. Phytochemical analyses reveal a broad range of bioactive metabolites, including flavonoids (luteolin, quercetin glycosides), phenolic acids (chicoric, chlorogenic, caffeic acids), terpenoids (taraxasterol, taraxerol), sesquiterpene lactones (taraxinic acid β-D-glucopyranosyl ester), and phytosterols (β-sitosterol, cycloartenol), distributed throughout the leaves, roots, flowers, and latex. These compounds are believed to underlie the plant's wide spectrum of pharmacological activities.

Taraxacum officinale belongs to the Asteraceae family and has been widely used in traditional medicine for centuries. The name derives from the Greek "taraxos" (disorder) and "akos" (remedy), reflecting its curative reputation, while "officinale" denotes its medicinal significance [7]. This perennial herb, recognized by its rosette leaf structure, yellow flowers, and milky latex, is native to temperate regions of Europe, Asia, and North America but is now globally distributed [8,9].

Traditionally, dandelion has been used as a diuretic, liver tonic, and digestive aid. Modern pharmacological studies confirm its broad biological activities, including antidiabetic, antioxidant, hepatoprotective, anti-inflammatory, neuroprotecti with its bioactive constituents.

Anticancer potential, attributed to its rich composition of bioactive phytochemicals. Its extracts and isolated compounds exhibit cytotoxic, pro-apoptotic, and gene-modulating effects against various cancer cell lines, while showing minimal toxicity toward normal cells. These characteristics make dandelion an attractive

source for developing adjunct natural therapies in oncology. Nevertheless, further standardized preclinical and clinical studies are necessary to confirm efficacy, safety, and bioavailability of its active components.

Distribution of Phytochemicals in *Taraxacum officinale*

Taraxacum officinale is a rich source of bioactive compounds with diverse pharmacological properties, including alkaloids, saponins, flavonoids, glycosides, phenols, tannins, terpenoids, steroids, anthracenosides, phlobatannins, and anthraquinones. These secondary metabolites are distributed differently throughout the plant's parts – leaves, roots, flowers and stems – contributing to its therapeutic activities [10-15] .

Phytochemical studies have identified numerous active constituents in different tissues of the plant, including: phenolic acids (Cinnamic acid, sinapic acid, ferulic acid, monocaffeoyltartaric acid, dicaffeoylquinic acid, and chicoric acid), flavonoids (Quercetin glycoside, hesperidin, naringenin, kaempferol, luteolin diglycoside, luteolin-7-glycoside, luteolin glycoside II and luteolin), terpenoids and steroids (Cycloartenol, taraxerol, ψ-taraxasterol, taraxacolide-β-D-glucoside, phytol, lupeol, taraxasteryl acetate and cycloartenol acetate), and polysaccharides (inulin, a prebiotic compound widely used in the food industry for improving the texture of functional food products) [15-22] .

The latex extracted from *T. officinale* contains various bioactive compounds, including taraxinic acid, β-D-glucopyranosyl ester, α-amyrin acetate, β-amyrin acetate, cycloartenol acetate, and lupeol acetate [23].

In terms of anticancer activity, several compounds with oncological potential have been identified in this plant, including taraxasterol, taraxinic acid, and taraxinic acid β-D-glucopyranosyl ester, which exhibit promising effects against different types of cancer [24].

Anticancer Effects of *Taraxacum officinale* Extracts

Different parts of *Taraxacum officinale*—including its leaves, roots, and flowers—have been extensively studied for their cytotoxic and antitumor potential using extracts prepared with various solvents.

Aqueous extracts of *T. officinale* have shown potent antitumor activity against fourteen distinct cancer cell lines, such as cervical carcinoma (HeLa), breast cancer (MCF-7, SK-BR-3, MDA-MB-231), endometrial carcinoma (Ishikawa, AN3 Ca), ovarian cancer (OVCAR-3, SKOV-3), bladder carcinoma (EJ28, RT112), colon cancer (SW620), pancreatic carcinoma (PANC-1), hepatocellular carcinoma (HuH-7), and lung cancer (A549). Treatment markedly decreased cancer cell viability, with IC_{50} values ranging from 12 to 160 mg/mL, and induced apoptosis, inhibited cell migration, and disrupted mitochondrial function, particularly in ovarian cancer models. Importantly, normal human fibroblasts (NHDF-C) were not affected, indicating the extract's selective cytotoxicity toward malignant cells [25].

Polysaccharides isolated from *T. officinale* also demonstrated significant antiproliferative activity, reducing the growth of human hepatocellular carcinoma cells (HepG2) by about 52% at higher concentrations (2,000 µg/mL) [26]. Similarly, aqueous extracts from different plant parts inhibited several cancer cell lines—including HepG2, MCF-7/AZ, LNCaP C4-2B, Calu-6, HCT-116, and SNU-601—in a dose- and time-dependent manner [27].

At 200 µg/mL, cell viability decreased by approximately 40–50% after 48 hours of treatment. Moreover, an increased release of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1-alpha (IL-1α), was observed. This immunomodulatory response suggests that *T. officinale* may exert antitumor effects not only by directly inhibiting tumor cell proliferation but also by stimulating the immune system's defense against cancer [28].

Anticancer Activity of *Taraxacum officinale* Extracts

Extracts derived from various parts of *Taraxacum officinale* have demonstrated marked cytotoxic and antitumor activities against multiple cancer types through diverse mechanisms, including inhibition of cell proliferation, induction of apoptosis, and regulation of gene expression involved in tumor progression [29].

Non-polar solvent extracts, such as those obtained with methylene chloride, displayed higher cytotoxic potency against oral squamous carcinoma cells (SGT), achieving up to 97% growth inhibition at 200 µg/mL. In contrast, methanolic and aqueous extracts—rich in hydrophilic constituents such as chicoric acid and polysaccharides—exhibited variable potency, suggesting that solvent polarity significantly influences the extraction of active compounds.

Ethanolic flower extracts showed a dose-dependent antiproliferative effect on ovarian cancer cells (SK-OV-3) within a concentration range of 1.56–100 µg/mL. The treatment induced cell cycle arrest at the S and G_2/M phases, followed by apoptosis and DNA fragmentation in the sub-G_0/G_1 phase. Activation of the tumor

suppressor gene *p53* and upregulation of pro-apoptotic protein Bax, accompanied by decreased anti-apoptotic Bcl-2 expression, confirmed the apoptotic mechanism [30,31].

The biological model used also influences the outcome. *In vitro* assays on HepG2 and HL-60 cells revealed strong cytotoxicity mediated by apoptosis and DNA fragmentation, while *in vivo* studies in DMBA-induced breast cancer models showed regulation of genes such as *Pdk1*, *Akt1*, and *Erbb2*, indicating a multi-target mode of action.

Fractionation studies demonstrated that the methylene chloride extract exerted superior cytotoxicity (97% inhibition at 200 µg/mL) compared to hexane, ethyl acetate, butanol, and methanol fractions when tested on oral cancer cells (SGT) [83]. Furthermore, ethanolic leaf extracts triggered apoptosis in cervical carcinoma stem cells by increasing *RARβ2*gene expression—both retinoic acid–dependent and –independent—while downregulating *Sox2*, a gene critical for cancer cell self-renewal.

A methanolic extract was also found to sensitize hepatocellular carcinoma (Huh7) cells to TRAIL (Tumor Necrosis Factor–Related Apoptosis–Inducing Ligand). Combined treatment reduced cell viability by 52%, activating apoptosis via inhibition of MKK7–TIPRL interaction and stimulation of MKK7–JNK phosphorylation. Chicoric acid reproduced similar results, confirming the involvement of the JNK signaling pathway [32].

Root extracts of *T. officinale* inhibited proliferation, migration, and invasion of gastric carcinoma cells (SGC7901 and BGC823) without affecting normal gastric epithelial cells (GES-1). These effects were linked to downregulation of the long non-coding RNA *CCAT1*, a gene associated with colorectal carcinogenesis [80].

Differences in cytotoxicity among plant parts were evident: root extracts rich in inulin, phenolic acids, and taraxinic derivatives differed in potency from aerial parts and latex, which contain higher terpenoid and flavonoid levels. For example, stem extracts showed stronger cytotoxicity against HL-60 leukemia cells (IC_{50} = 71 µg/mL) compared with leaf extracts (IC_{50} = 540 µg/mL), consistent with compound distribution variations [33].

Biosynthesized silver nanoparticles (TOL-AgNPs) from aqueous leaf extracts exhibited enhanced anticancer activity, inhibiting up to 95% of HepG2 cell proliferation at 200 µg/mL [37]. Similarly, whole-plant extracts displayed strong cytotoxicity in breast cancer cells (MCF-7), with an IC_{50} of 190.5 µg/mL [34].

Ethanolic fruit extracts demonstrated protective antioxidant effects in rat brain tissue, counteracting sodium nitroprusside–induced cytotoxicity and lipid peroxidation at 1–20 µg/mL. The extract also showed strong radical scavenging activity against DPPH, NO, and H_2O_2, preventing Fe^{2+}-induced oxidative damage [35].

In *in vivo* experiments, aqueous root extracts reduced serum CA15-3 levels in DMBA-induced breast cancer in female mice. Expression of genes such as *Pdk1*, *Akt1*, *Pik3r1*, *Map3k1*, *Erbb2*, and *Plk3ca*—which were initially upregulated by DMBA—was normalized following treatment, except for *Pik3r1*, which increased. Histological analysis revealed reduced Bcl-2 expression in mammary ducts and acini, confirming apoptosis activation [38,39].

Together, these findings underline the versatility of *T. officinale* extracts, whose anticancer effects are influenced by plant part, extraction solvent, and molecular formulation.

Anticancer Activity of Bioactive Compounds Isolated from *Taraxacum officinale*

The anticancer potential of *T. officinale* is largely attributed to its bioactive constituents. Taraxasterol, one of its main triterpenes, inhibits tumor proliferation and invasion, particularly in colon, liver, and breast cancers. It induces G_0/G_1 cell cycle arrest, upregulates *Hint1* and *Bax*, downregulates *Bcl-2* and cyclin D1, and promotes demethylation of the *Hint1* promoter, restoring tumor suppressor function [40].

Taraxasterol also suppresses papillary thyroid cancer cell migration by preventing TGF-β–induced epithelial-mesenchymal transition (EMT). This effect is mediated through inhibition of MMP-2 and MMP-9 and blockade of the Wnt/β-catenin signaling pathway [41].

Taraxinic acid demonstrated antiproliferative activity in human HL-60 leukemia cells by promoting differentiation, reducing *c-Myc* oncogene expression, and increasing CDK inhibitors *p21* and *p27*, suggesting therapeutic relevance in leukemia [42].

β-sitosterol, a major phytosterol, inhibited cervical carcinoma cell proliferation (CaSki and HeLa) by upregulating *p53*and downregulating HPV *E6*, a viral oncogene associated with cervical cancer progression [43].

Chlorogenic acid suppressed hepatocellular carcinoma (HepG2) proliferation through inhibition of ERK1/2 signaling and induced apoptosis in osteosarcoma cells (U2OS, Saos-2, MG-63) via the same pathway [93]. Likewise, chicoric acid reduced gastric cancer cell viability (SGC7901, MGC803) in a dose-dependent manner (5–100 µM) by activating AMPK and inducing autophagy [44].

Conclusions

Collectively, existing evidence demonstrates that *Taraxacum officinale* exerts its anticancer effects through multifaceted mechanisms, including inhibition of proliferation, induction of apoptosis, disruption of

mitochondrial function, modulation of inflammatory mediators, and regulation of key oncogenic signaling pathways such as PI3K/Akt and JNK. Importantly, most studies emphasize the plant's selectivity—targeting cancer cells while sparing normal cells—which represents a crucial advantage for reducing treatment-associated toxicity.

Beyond its anticancer action, the plant's antioxidant, anti-inflammatory, hepatoprotective, and immunomodulatory activities enhance its therapeutic value. Its low toxicity and accessibility support its consideration as a complementary or adjuvant option in cancer therapy.

However, translating these promising preclinical results into clinical application requires standardized extraction methods, detailed pharmacokinetic studies, and well-designed clinical trials to validate efficacy and safety.

In summary, *Taraxacum officinale* emerges as a promising source of multifunctional bioactive compounds with significant potential in developing safe, affordable, and plant-based anticancer therapies.

Reference

1. https://www.who.int/news-room/fact-sheets/detail/cancer
2. https://insp.gov.ro/2024/07/08/profil-de-tara-privind-cancerul-2023/
3. *Shaikh, A. M., et al. "Medicinal plants as potential source of anticancer agents: a review." Journal of Pharmacognosy and Phytochemistry 5.2 (2016): 291-295.*
4. *Martinez, M., et al. "Taraxacum officinale and related species—An ethnopharmacological review and its potential as a commercial medicinal plant." Journal of Ethnopharmacology 169 (2015): 244-262.*
5. *Devi, J. Renuka, and E. Berla Thangam. "Mechanisms of anticancer activity of sulforaphane from Brassica oleracea in HEp-2 human epithelial carcinoma cell line." Asian Pacific Journal of Cancer Prevention 13.5 (2012): 2095-2100.*
6. *Najmi, Asim, et al. "Modern approaches in the discovery and development of plant-based natural products and their analogues as potential therapeutic agents." Molecules 27.2 (2022): 349.*
7. *Di Napoli, Agnese, and Pietro Zucchetti. "A comprehensive review of the benefits of Taraxacum officinale on human health." Bulletin of the National Research Centre 45.1 (2021): 110.*
8. *Grauso, Laura, et al. "Common dandelion: A review of its botanical, phytochemical and pharmacological profiles." Phytochemistry Reviews 18.4 (2019): 1115-1132.*
9. *Lwin, Myat Kay Thwe. "Study on botanical characters and preliminary chemical composition of dandelion leaves Taraxacum officinale FH Wigg." Proceedings of the 2nd Myanmar-Korea Conference Research Journal, Yangon, Mjanma. 2019.*
10. *Schütz, Katrin, Reinhold Carle, and Andreas Schieber. "Taraxacum—a review on its phytochemical and pharmacological profile." Journal of ethnopharmacology 107.3 (2006): 313-323.*
11. *Oseni, L. A., and I. Yussif. "Screening ethanolic and aqueous leaf extracts of Taraxacum offinale for in vitro bacteria growth inhibition." (2012).*
12. *Ghaima, Kais Kassim, Noor Makie Hashim, and Safaa Abdalrasool Ali. "Antibacterial and antioxidant activities of ethyl acetate extract of nettle (Urtica dioica) and dandelion (Taraxacum officinale)." Journal of Applied Pharmaceutical Science 3.5 (2013): 096-099.*
13. *Singh, Avinash, Ramesh Chandra, and Mohd Nayeem Ali. "Rate of extraction and phytochemical screening of selected medicinal herbs for herbal yoghurt." The Pharma Innovation 4.3, Part A (2015): 15.*
14. *Bylka, W., I. Matlawska, and R. Frański. "Essential oil composition of Taraxacum officinale." Acta Physiologiae Plantarum 32 (2010): 231-234.*
15. *Cortés, Natalie, et al. "Microscopical descriptions and chemical analysis by HPTLC of Taraxacum officinale in comparison to Hypochaeris radicata: a solution for mis-identification." Revista Brasileira de Farmacognosia 24.4 (2014): 381-388.*
16. *Wirngo, Fonyuy E., Max N. Lambert, and Per B. Jeppesen. "The physiological effects of dandelion (Taraxacum officinale) in type 2 diabetes." The review of diabetic studies: RDS 13.2-3 (2016): 113.*
17. *Ivanov, Ivan, et al. "GC-MS characterization of n-hexane soluble fraction from dandelion (Taraxacum officinale Weber ex FH Wigg.) aerial parts and its antioxidant and antimicrobial properties." Zeitschrift für Naturforschung C 73.1-2 (2018): 41-47.*
18. *KHAN, FAHAD SAID, et al. "Phytochemical screening of three traditional medicinal plants: taraxacum officinale, geranium wallichianum, and elaeagnus parvifolia." Pak. J. Bot 56.1 (2024): 239-246.*
19. *Cheraghipour, Kourosh, et al. "Phytochemical screening, protoscolicidal activity and mechanisms of action of Taraxacum officinale extract against hydatid cyst protoscoleces." Archives of Razi Institute 79.6 (2024): 1311-1317.*

20. *Bashir, Shaista, and Latif Ahmad Peer. "Phytochemistry, biological properties, economic and ecological importance of Taraxacum officinale, A review." Int J Botany Stud 7.2 (2022): 574-582.*
21. *Huber, Meret, et al. "Identification, quantification, spatiotemporal distribution and genetic variation of major latex secondary metabolites in the common dandelion (Taraxacum officinale agg.)." Phytochemistry 115 (2015): 89-98.*
22. *Bashir, Shaista, and Latif Ahmad Peer. "Phytochemistry, biological properties, economic and ecological importance of Taraxacum officinale, A review." Int J Botany Stud 7.2 (2022): 574-582.*
23. *Obafemi, Olabisi Tajudeen, et al. "Pharmacological relevance of taraxasterol: A review." Pharmacological Research-Modern Chinese Medicine (2024): 100533.*
24. *Yan, Qingzi, et al. "The phytochemical and pharmacological profile of dandelion." Biomedicine & Pharmacotherapy 179 (2024): 117334.*
25. *Menke, K., et al. "Taraxacum officinale extract induces antitumorigenic effects in ovarian carcinoma cell lines." European Journal of Gynaecological Oncology 40.1 (2019).*
26. *Chen, Xiaoyu, et al. "Optimization of extraction process from Taraxacum officinale polysaccharide and its purification, structural characterization, antioxidant and anti-tumor activity." Journal of Food Measurement and Characterization 14 (2020): 194-206.*
27. *Modaresi, Mehrdad, and Narges Resalatpour. "The effect of Taraxacum officinale hydroalcoholic extract on blood cells in mice." Advances in hematology 2012.1 (2012): 653412.*
28. *Lee, H. H.. "Cytotoxic and Antioxidant Effects of Taraxacum coreanum Nakai. and T. officinale WEB. Extracts." The Korean Journal of Medicinal Crop Science 16 (2008): 79-85.*
29. *Choi, Eun-Jeong, and Gun-Hee Kim. "Dandelion (Taraxacum officinale) flower ethanol extract inhibits cell proliferation and induces apoptosis in human ovarian cancer SK-OV-3 cells." Food science and biotechnology 18.2 (2009): 552-555.*
30. *Nassan, Mohamed Abdo, et al. "Effect of Taraxacum officinale extract on PI3K/Akt pathway in DMBA-induced breast cancer in albino rats." Bioscience reports 38.6 (2018): BSR20180334.*
31. *Colle D, Arantes LP, Rauber R, et al. Antioxidant properties of Taraxacum officinale fruit extract are involved in the protective effect against cellular death induced by sodium nitroprusside in brain of rats. Pharm Biol. 2012;50(7):883-891.*
32. *Saratale RG, Benelli G, Kumar G, Kim DS, Saratale GD. Bio-fabrication of silver nanoparticles using the leaf extract of an ancient herbal medicine, dandelion (Taraxacum officinale), evaluation of their antioxidant, anticancer potential, and antimicrobial activity against phytopathogens. Environ Sci Pollut Res Int. 2018;25(11):10392-10406.*
33. *Yoon JY, Cho HS, Lee JJ, et al. Novel TRAIL sensitizer Taraxacum officinale F.H. Wigg enhances TRAIL-induced apoptosis in Huh7 cells. Mol Carcinog. 2016;55(4):387-396.*
34. *Ahmadi S, Saberivand A, Jalili C, Asadpour R, Khordadmehr M, Saberivand M. Hydroalcoholic extract of Taraxacum officinaleinduces apoptosis and autophagy in 4T1 breast cancer cells. Vet Res Forum. 2023,14(9):507-513.*
35. *Ahmadi S, Saberivand A, Jalili C, Asadpour R, Khordadmehr M, Saberivand M. Hydroalcoholic extract of Taraxacum officinaleinduces apoptosis and autophagy in 4T1 breast cancer cells. Vet Res Forum. 2023;14(9):507-513.*
36. *Fan, M.; Zhang, X.; Song, H.; Zhang, Y. Dandelion (Taraxacum Genus): A Review of Chemical Constituents and Pharmacological Effects. Molecules 2023, 28, 5022.*
37. *Yoon, Ji-Yong, et al. "Novel TRAIL sensitizer Taraxacum officinale FH Wigg enhances TRAIL-induced apoptosis in Huh7 cells." Molecular carcinogenesis 55.4 (2016): 387-396.*
38. *Appiah-Opong, Regina, et al. "Antiproliferative, antioxidant activities and apoptosis induction by Morinda lucida and Taraxacum officinale in human HL-60 leukemia cells." Journal of Global Biosciences 5.7 (2016): 4281-4291.*
39. *Degan, Seamus E., and Irwin H. Gelman. "Emerging roles for AKT isoform preference in cancer progression pathways." Molecular Cancer Research 19.8 (2021): 1251-1257.*
40. *Halilovic, Ensar, et al. "PIK3CA mutation uncouples tumor growth and cyclin D1 regulation from MEK/ERK and mutant KRAS signaling." Cancer research 70.17 (2010): 6804-6814.*
41. *Cheng, Dali, Zhiqiang Guo, and Shulan Zhang. "Effect of β-sitosterol on the expression of HPV E6 and p53 in cervical carcinoma cells." Contemporary Oncology/Współczesna Onkologia 19.1 (2015): 36-42.*
42. *Esatbeyoglu, Tuba, et al. "Sesquiterpene lactone composition and cellular Nrf2 induction of Taraxacum officinale leaves and roots and taraxinic acid β-d-glucopyranosyl ester." Journal of medicinal food 20.1 (2017): 71-78.*
43. *Sapio, Luigi, et al. "Chlorogenic acid activates ERK1/2 and inhibits proliferation of osteosarcoma cells." Journal of cellular physiology 235.4 (2020): 3741-3752.*

44. *Choi, Jung-Hye, et al. "Taraxinic acid, a hydrolysate of sesquiterpene lactone glycoside from the Taraxacum coreanum N AKAI, induces the differentiation of human acute promyelocytic leukemia HL-60 Cells." Biological and Pharmaceutical Bulletin 25.11 (2002): 1446-1450.*
45. *Sapio, Luigi, et al. "Chlorogenic acid activates ERK1/2 and inhibits proliferation of osteosarcoma cells." Journal of cellular physiology 235.4 (2020): 3741-3752.*
46. *Sun, Xiaoli, et al. "Chicoric acid (CA) induces autophagy in gastric cancer through promoting endoplasmic reticulum (ER) stress regulated by AMPK." Biomedicine & Pharmacotherapy 118 (2019): 109144.*
47. *Yan, Yuan, et al. "Chlorogenic acid inhibits hepatocellular carcinoma in vitro and in vivo." The Journal of Nutritional Biochemistry 46 (2017): 68-73.*

PRINTED OR MILLED? A SIDE-BY-SIDE LOOK AT SURGICAL GUIDES FOR DENTAL IMPLANTS

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DOI: https://doi.org/10.66793/titum19proceeding12

ABSTRACT

Computer-guided surgery has transformed dental implantology by improving accuracy and safety through the use of digitally planned surgical guides. This review compares two fabrication techniques, namely additive manufacturing (3D-printing) and subtractive manufacturing (milling), in terms of accuracy, cost, clinical outcomes, patient satisfaction, and workflow efficiency. A comprehensive literature search identified studies and reviews directly comparing 3D-printed and CAD/CAM-milled surgical guides. Both methods demonstrated comparable dimensional accuracy, with reported deviations typically within 1–2 mm at the implant apex (within clinically acceptable limits). Some studies found milled surgical guides slightly more consistent and precise, while others reported no statistically significant differences. Cost analyses revealed a substantial advantage for 3D printing due to lower material and equipment expenses, facilitating wider clinical adoption. Both guide types produced similar implant survival rates and postoperative comfort, confirming that clinical outcomes and patient satisfaction are influenced more by the guided approach itself than by the fabrication method of the surgical guide. From a practical standpoint, 3D-printing offers greater flexibility and lower cost, while milling provides robust consistency and material strength. Overall, both technologies ensure accurate, predictable implant placement.

INTRODUCTION

Dental implant placement has been revolutionized by the use of computer-guided surgical guides, which enhance accuracy and safety compared to freehand surgery. By using preoperative cone-beam computed tomography (CBCT) and digital implant planning, surgical guides help avoid critical anatomic structures and mispositioned implants, thereby reducing complications such as nerve injury, sinus perforation, or improper angulation. This leads to shorter surgeries, reduced anxiety and pain for the patient, and overall improved clinical outcomes. At the moment, two main fabrication technologies are used to produce these guides: additive manufacturing (3D printing) and subtractive manufacturing (milling). Both methods rely on the same digital planning data but differ in how the physical guide is created. This review aims to compare 3D-printed and CAD/CAM milled surgical guides for dental implant placement in terms of accuracy, cost, clinical outcomes, patient satisfaction, and manufacturing workflow.

Historically, stereolithographic 3D printing (a form of rapid prototyping) has been widely used to fabricate implant guides, while newer CAD/CAM milling techniques have also been developed. Each method has potential advantages: 3D printing allows complex shapes and in-house production at low cost, whereas milling can produce guides with high material density and may avoid some data conversion errors by using coordinated fabrication methods. Given the growing adoption of in-office digital dentistry equipment, clinicians are interested in whether one method offers superior outcomes or efficiency over the other. This review gathered data from recent studies on guided implant surgery to evaluate differences between 3D-printed surgical guides and milled surgical guides.

MATERIALS AND METHODS

A literature review was conducted to compare 3D-printed surgical guides and milled surgical guides for dental implants. An electronic search of the relevant journals was performed using keywords such as “3D printed implant guide”, “milled CAD/CAM surgical template”, “accuracy”, “cost”, “clinical outcome”, and “patient satisfaction”. Inclusion criteria focused on studies that directly compared guides fabricated by 3D-printing technique versus milling technique, as well as high-quality reviews or meta-analyses on guided implant surgery.

Key data were extracted on accuracy, cost factors, clinical outcomes (implant success and complications), patient-related outcomes (satisfaction or comfort), and manufacturing workflow differences. Both in vitro accuracy studies and clinical trials were considered. The search revealed multiple in vitro comparative studies[6,7], cost analyses from industry reports[3], and clinical evaluations of guided surgery outcomes[2,7].

RESULTS

Accuracy

Dimensional accuracy of implant placement using 3D-printed surgical guides vs milled surgical guides has been investigated in several studies. Overall, both fabrication methods achieve comparable accuracy in translating virtual implant plans to the patient. For example, a 2020 pilot clinical trial using CNC-milled guides in edentulous jaws reported average deviations of ~1.5 mm at the implant apex with no complications, and concluded that milled guides provided accuracy comparable to stereolithographic (SLA) 3D-printed surgical guides[2]. Similarly, an in vitro study by Mukai et al. (2021) found no statistically significant difference in overall guide precision between milled and 3D-printed guides[7]. The average deviations in guide fit or implant transfer accuracy were on the order of a few tenths of a millimeter for both methods[1,4], which is generally within clinically acceptable limits.

Some differences have been noted in specific accuracy parameters. Abduo and Lau (2020) observed that milled guides were more accurate than printed guides on certain measurements, including internal surface fit and sleeve position, with milled templates showing significantly less vertical and horizontal deviation at sleeve midpoints[6]. This suggests milling may have a slight edge in dimensional precision of the guide, potentially due to the elimination of layer-printing artifacts. Additionally, one study reported that while the average errors of milled and printed surgical guides were similar, 3D-printed guides showed greater variance in errors (higher coefficient of variation) than milled guides[5]. In other words, milling produced more consistent results across samples, whereas printed guides had slightly more variability in accuracy. However, other research does not corroborate a significant accuracy gap. Park et al. (2014) directly compared five-axis milled surgical guides to SLA-printed surgical guides and found no significant difference in the implant placement errors between the two fabrication techniques[17]. They reported average horizontal and vertical deviations of approximately 0.14–0.20 mm for milled surgical guides, which were comparable to those of the printed surgical guides[1].

Cost

Cost efficiency is a key distinction between 3D printing and milling for surgical guides. 3D-printed guides generally offer a significant cost advantage over traditional lab-fabricated or milled guides. The materials for resin printing are less expensive per guide, and desktop 3D printers are relatively affordable for dental practices. 3D-printed guides are substantially more cost-effective on a per-case basis. The cost difference can be hundreds of dollars less for a printed guide relative to a milled guide[3]. Koch et al. (2018) demonstrated that surgical templates could be fabricated with low-cost 3D printers without sacrificing accuracy, highlighting the cost-effectiveness of additive manufacturing for guides[6]. The lower material cost of printed guides enables wider adoption of guided surgery, especially in practices that found the high price of commercial milled guides prohibitive[3].

Milled guides, on the other hand, involve higher material and production costs. Milling a guide typically requires specialized acrylic or polymer blocks and the use of an expensive milling machine or a fee to a milling center. These machines and materials entail substantial upfront and maintenance costs, which are reflected in the higher price of each milled surgical guide. Milling also inherently produces waste (since material is cut away) and may require tools (burs) that add to the expense. For clinicians who do not own a milling unit, ordering a CAD/CAM milled guide from a lab incurs laboratory fees that exceed the cost of printing. As a result, cost considerations often drive clinicians toward 3D printing for guide fabrication[3]. In fact, the affordability of in-house 3D printing has been cited as a reason guided implant surgery is becoming more accessible and routine in smaller dental offices[3]. It should be noted that while printing is cheaper per guide, there is still an initial investment in a printer and resin curing equipment; however, this cost is recouped quickly if surgical guides are used regularly due to the high cost of alternatives[3]. Milling equipment, by comparison, has a higher cost barrier to entry for

Clinics aiming to adopt guided surgery widely often find that investing in a 3D printing workflow represents a better return on investment than relying on third-party milled guides[3]. Milling may still be preferred in certain scenarios (for example, if an in-house milling system is already available or if extremely

high precision for complex cases is demanded), but from a cost perspective, additive manufacturing is generally the more cost- efficient choice.

Clinical Outcomes

Both 3D-printed and milled surgical guides facilitate improved clinical outcomes in implant surgery by enabling more precise implant placement compared to freehand techniques. The use of any digitally planned guide, regardless of fabrication method, helps reduce surgical errors and complications, which can translate to better long-term results. For instance, guided implant placement has been associated with lower risk of damage to vital structures, more accurate prosthetic positioning, and reduced need for corrective surgeries[3,7]. By improving implant positioning, guides also contribute to optimal load distribution and may reduce biomechanical complications over time[7].

Studies of guided surgery consistently report high implant survival rates and low complication rates when using guides. Chai et al. (2020) placed 44 implants with milled guides in edentulous jaws and noted 100% implant survival with no surgical or immediate prosthetic complications in their series[2]. Similar success can be achieved with 3D-printed guides, as accuracy of placement is comparable. Current evidence does not indicate any difference in long-term implant success between 3D-printed and milled guides, since the critical factor is accurate implant positioning, which both methods achieve. No direct clinical trials have reported divergent implant survival or osseointegration outcomes attributable to the guide fabrication technique. In other words, a properly planned and fabricated guide; whether milled or 3D-printed; should recreate the intended implant positioning and thereby a similar clinical result.

Postoperative morbidity and patient recovery also tend to be improved with guided surgery (versus freehand), but they are not meaningfully different when comparing 3D-printed surgical guides with milled surgical guides. The surgical invasiveness is reduced in guided procedures (due to smaller incisions or flapless approaches and shorter surgery duration), which leads to less postoperative pain and swelling[7]. Both 3D-printed and milled surgical guides equally enable these minimally invasive techniques. There is no indication that one type of guide causes different tissue healing or infection rates than the other, as both use biocompatible guide materials that are safe for medical use.

Patient Satisfaction

Using a surgical guide generally enhances patient satisfaction with the implant treatment experience, and this benefit applies to both 3D-printed and milled guides. Guided surgery typically results in shorter procedure times and less invasive surgery, which patients appreciate[3]. For example, reports have documented that complex surgeries requiring multiple implants can be simplified with surgical guides, reducing the surgery time significantly (for example, a case that would normally take two hours reduced to 30 minutes with a dual-guide approach)[3]. A faster, smoother surgery means patients spend less time under anesthesia or less chair time and often have easier recoveries. The precision of the surgical guides also reduces the likelihood of unexpected intraoperative events (such as the need to adjust implant position or increase the length of an incision), which in turn lowers patient anxiety and discomfort[7]. Patients often experience less pain and swelling postoperatively when a surgical guide is used because tissue handling is more precise and flapless techniques can be employed[7]. These factors contribute to higher patient satisfaction and willingness to undergo implant procedures with guided technology.

When comparing 3D-printed vs milled guides from the perspective of the patient, there is no notable difference in comfort or satisfaction as long as the surgical guide fits well. Both types of guides serve the same function during surgery and are made of similar rigid biocompatible materials. The fit and stability of the guide in the mouth are critical to patient comfort: a well-fitted surgical guide (either printed or milled) will be only minimally bulky and will stay stable during the procedure. Studies indicate that a properly designed guide does not cause significant discomfort regardless of fabrication method[7]. If anything, differences might arise from surface texture or finish (a milled guide may have a very smooth internal surface, whereas a 3D-printed guide has a layered texture) but in practice surgical guides are usually polished/finished and any minor texture differences are not perceivable by the patient. Both types can be adjusted or relieved if pressure points exist, ensuring comfort.

Manufacturing Workflow

From a practical standpoint, 3D-printing offers greater flexibility and lower barriers to entry. A dentist with a moderate investment can set up a small 3D printing lab and fabricate guides on demand, integrating with digital scans from an intraoral scanner and planning software[3]. The workflow is highly digital and can be streamlined: for example, after virtual planning, the guide STL can be printed overnight and ready the next morning for surgery. This decentralization means even clinics without access to large milling machines can adopt guided surgery. Milling workflows are often tied to specific systems (for example, certain implant companies or software provide a closed-loop from planning to milling). In-office milling of surgical guides is less common than printing because the equipment is costly and often the size/geometry of surgical guides pushes the limits of chairside mills. Some integrated systems (such as CEREC Guide in early iterations) milled guides with a limited approach (for example, one hole at a time in a standardized acrylic block), but many of these have since shifted to 3D-printing for more complex or full-arch guides.

Another consideration is material and design limitations. Milled surgical guides are constrained by the size of the milling blank and the tool diameter – they might have slightly thicker features or require support bars during milling to prevent movement. Intricate design features like long protruding sleeves or extremely thin windows might be challenging to mill. 3D printing, conversely, can fabricate very complex shapes including hollow lattice structures or delicate features, as long as they are supported during the print process. This means printing can accommodate innovative guide designs (such as stackable surgical guides, or surgical guides with integrated soft tissue supports) with relative ease, whereas milling might not. On the other hand, the solid block material used for milling can be very sturdy and not prone to micro-porosity, whereas printed guides need adequate post-curing to achieve full strength and can be sensitive to resin curing protocols.

DISCUSSION

The comparison of 3D-printed versus milled implant surgical guides reveals that both methods are highly effective, with each having particular strengths. Accuracy outcomes from the literature indicate that while some differences can be measured in vitro, these differences are generally small and unlikely to be clinically significant. Milled guides have been reported in isolated studies to exhibit slightly superior accuracy on certain metrics (such as marginal fit and sleeve positioning)[6], but multiple other studies and reviews find no meaningful discrepancy[7,8]. In practice, both techniques reliably translate digital implant plans to the surgical field with sub-millimeter precision. Instead, the experience of the operator, the guide support type (teeth vs mucosa), and the quality of the planning process may have a larger impact on accuracy and success than the fabrication method itself[7]. Clinicians should ensure proper case selection (for example, using anchor pins or extra stabilization for fully edentulous cases) and adhere to best practices in guide design regardless of whether printing or milling is used.

When considering cost and practical implementation, 3D-printing emerges as a more feasible option for many dental practices. Its cost advantages[3] and flexibility have likely contributed to the democratization of guided implant surgery. The results of this review support that, since the clinical outcomes and accuracy are comparable between 3D-printed and milled surgical guides, the method that can deliver those results more economically and conveniently (often 3D-printing) will be the preferred choice in most scenarios.

However, milling has unique advantages that deserve consideration. The slightly better consistency (less variance) in accuracy of the milled surgical guides[5] could be beneficial in extremely precision-sensitive cases, though it remains unclear if this theoretical benefit reveals noticeable clinical differences. Additionally, milling avoids the layering process of printing, so issues like anisotropic shrinkage or layer delamination do not occur. In situations where absolute rigidity and accuracy are extremely important (for example, certain guided protocols for full-arch with immediate loading) some practitioners might trust the material properties of a milled surgical guide (solid block, potentially fewer microstructural flaws) over a printed one. That said, modern 3D-printed resins (especially when printed with high-quality machines and properly cured) have proven very robust and accurate, as evidenced by thousands of successful guided surgeries worldwide[3].

Another aspect discussed is the manufacturing workflow and turnaround time. In an urgent or same-day implant placement scenario, an in-office milled guide might actually be faster if the equipment is on hand; for instance, milling a small guide could be done in under an hour, whereas even a fast 3D print might take a couple of hours plus post-processing. Yet, not many clinics have the milling capability for guides, whereas an increasing number have a 3D printer. For routine cases, printing a surgical guide a day or two in advance of surgery is straightforward and fits well into digital workflows that already include intraoral scanning and virtual

planning. The ease of design iteration with printing (simply edit the STL and reprint) is also an advantage when adjustments are needed. Milling, in contrast, might require starting over with a new block if a design change is made, which is less material-efficient.

The literature on patient-centered outcomes specifically comparing these methods is uncommon; likely because patients cannot tell the difference during use. Our review infers that since both 3D-printed and milled surgical guides enable a guided approach, patient outcomes (pain, satisfaction) depend on guided vs non-guided technique rather than on the guide's fabrication method. Both methods have been successfully used in clinical practice with high patient acceptance.

One must also consider possible biases and limitations in the reviewed studies. Many accuracy studies are in vitro, using model simulations to measure deviations. These provide valuable insight but may not capture clinical handling differences. For example, a 3D-printed surgical guide might deform slightly if not thick enough, or a milled guide might require slightly different insertion technique; such nuances are hard to quantify on benchtop measurements but could affect clinical use. The systematic review by Lo Russo et al. (2023) highlighted significant heterogeneity in how accuracy is measured across studies (different definitions of "accuracy" like entry point deviation, apex deviation, angle error, etc., and varying reference points)[6]. This heterogeneity complicates direct comparisons and might explain why results seem conflicting. Standardized evaluation methods and more direct head-to-head clinical trials would be useful to further clarify if any clinically relevant differences exist between 3D-printed and milled surgical guides.

Future research could focus on the longevity and robustness of surgical guides from each method. For example, "how do printed vs milled guides hold up to sterilization and storage?". Preliminary data suggest that common printable dental resins, like autoclavable surgical guide resins, remain dimensionally stable through sterilization[3], and milled acrylic likewise is stable. But extreme temperatures or multiple sterilization cycles could potentially affect some printed resin guides, whereas milled PMMA might be more inert. These factors are minor for single-use guides (most surgical guides are discarded after the one-time surgery), but if a surgical guide needed to be reused or if surgeries are delayed, it could be worth examining.

Our discussion reinforces that the primary benefit is in using guided surgery whenever possible, rather than in the specific fabrication technique of the guide. Both 3D-printed and milled guides are mature technologies that enable accurate, safe implant placement. The differences lie in practical aspects: cost, convenience, and workflow. For most clinicians and patients, 3D-printing offers a convenient, low-cost solution without compromising accuracy or outcomes, which explains its growing popularity. Milled surgical guides remain a gold-standard option in certain contexts, backed by evidence of excellent accuracy and consistency, but their higher cost and logistical requirements limit widespread use.

CONCLUSIONS

3D-printed and milled surgical guides are both highly effective tools for improving the precision of dental implant placement, and the current evidence indicates that they result in comparable clinical outcomes. Both methods significantly enhance surgical accuracy, reduce the risk of complications, and improve patient experience compared to freehand implant surgery. The choice between 3D-printing and milling for surgical guide fabrication therefore comes down to practical considerations more than differences in efficacy. 3D-printing offers greater cost-efficiency and flexibility, enabling in-office production of surgical guides at a fraction of the cost compared to the milled surgical guides. It allows more clinicians to adopt guided surgery and tailor the workflow to their schedule. Milling, while more expensive, can provide highly consistent accuracy and utilizes a robust workflow that avoids some data conversion steps, making it a reliable option for those with access to the necessary equipment or for laboratories serving many clients.

From an accuracy standpoint, no clinically significant difference has been firmly established between the two fabrication methods. Both can deliver implant placements within acceptable deviation ranges when used properly. Therefore, clinicians can be confident in the guidance provided by either type of guide – the crucial factor is meticulous digital planning and proper surgical execution. In terms of patient outcomes, both 3D-printed and milled surgical guides contribute to high implant success rates and patient satisfaction through the shared mechanism of improved surgical precision.

In summary, 3D-printed vs milled guides represent two paths to the same goal: safer, more predictable implant surgery. Each has its advantages, with 3D-printing being the more cost-effective and accessible approach for most, and milling offering exceptional accuracy and a proven track record in specialized settings.

As digital dentistry continues to advance, we anticipate that both techniques will further improve, perhaps integrating features of each other (for example, hybrid workflows or new materials). For now, the evidence supports that either method can be employed without compromise to quality, allowing practitioners to choose the option that best fits their practice model and resources. The ultimate beneficiary is the patient, who receives the implant with minimal risk and maximal precision, regardless of how the guide was made.

CONFLICT OF INTEREST: I undersign, certificate that I do not have any financial or personal relationships that might bias the content of this work.

FINANCIAL SUPPORT: None to be declared.

AUTHOR'S CONTRIBUTIONS: All authors contributed equally to the manuscript.

REFERENCES

1. *Abduo, J., & Lau, D. (2020). Effect of Manufacturing Technique on the Accuracy of Surgical Guides for Static Computer-Aided Implant Surgery. International Journal of Oral & Maxillofacial Implants, 35(5), 931–938. DOI: 10.11607/jomi.7781;*
2. *Chai, J., Liu, X., Schweyen, R., Setz, J., Pan, S., Liu, J., & Zhou, Y. (2020). Accuracy of implant surgical guides fabricated using computer numerical control milling for edentulous jaws: a pilot clinical trial. BMC Oral Health, 20, 288. DOI: 10.1186/s12903-020-01283-4;*
3. *Formlabs Dental. (2017). Affordable In-House 3D Printed Surgical Guides. Formlabs Blog, October 10, 2017. (Industry report on guided surgery workflow and cost);*
4. *Hultin, M., Svensson, K. G., & Trulsson, M. (2012). Clinical advantages of computer-guided implant placement: a systematic review. Clinical Oral Implants Research, 23(Suppl 6), 124–135. DOI: 10.1111/j.1600-0501.2012.02545.x;*
5. *Koch, G., James, B., Gallucci, G., & Hamilton, A. (2018). Surgical Template Fabrication Using Cost-Effective 3D Printers. International Journal of Prosthodontics, 32(1), 97–100. DOI: 10.11607/ijp.5503;*
6. *Lo Russo, L., Pierluigi, M., Zhurakivska, K., Digregorio, C., Lo Muzio, E., & Laino, L. (2023). Three-Dimensional Accuracy of Surgical Guides for Static Computer-Aided Implant Surgery: A Systematic Review. Prosthesis, 5(3), 57 (Article 57). DOI: 10.3390/prosthesis5030057;*
7. *Mukai, S., Mukai, E., Santos-Júnior, J. A., Shibli, J. A., Faveri, M., & Giro, G. (2021). Assessment of the reproducibility and precision of milling and 3D printing surgical guides. BMC Oral Health, 21(1), 1. DOI: 10.1186/s12903-020-01363-5;*
8. *Park, J.-M., Yi, T.-K., Koak, J.-Y., Kim, S.-K., Park, E.-J., & Heo, S.-J. (2014). Comparison of five-axis milling and rapid prototyping for implant surgical templates. International Journal of Oral & Maxillofacial Implants, 29(2), 374–383. DOI: 10.11607/jomi.3265.*

AN ANALYSIS OF IMPACT, MECHANISMS, AND CONTROL MEASURES

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DOI: https://doi.org/10.66793/titum19proceeding13

Abstract

The paper highlights counterfeit medicines as a major global threat, affecting antibiotics, innovative biologics, and PDE5 inhibitors. Counterfeits lead to therapeutic failure, severe adverse reactions, and contribute to antimicrobial resistance. Cases such as Augmentin, Avastin, Eprex, Lantus, and semaglutide illustrate the scope of the problem and its serious clinical risks. Effective prevention requires international cooperation (WHO, INTERPOL, EMA), modern traceability technologies (GS1, DataMatrix, MAS), and public education. Ultimately, combating counterfeit medicines is essential for patient safety and maintaining trust in healthcare systems.

Key words: *counterfeit medicines; antibiotics; biological drugs; PDE5 inhibitors; antimicrobial resistance; patient safety; WHO; INTERPOL; traceability; serialization; public health*

1. Introduction

Over the past two decades, antibiotics have been among the most frequently counterfeited classes of medicines, as repeatedly reported by the WHO and other international regulatory authorities. Beyond individual clinical risks, counterfeit antibiotics have major public health consequences: they foster antimicrobial resistance and erode patients' trust in essential therapies.

The WHO has repeatedly issued alerts concerning falsified antibiotics, notably for the amoxicillin + clavulanic acid combi5tnation (Augmentin). In October 2019, two consecutive alerts drew attention to counterfeit batches found in Africa and Asia, some containing no active ingredients or substandard doses. The targeted products included *Amoxicillin + Clavulanate* (Medical Product Alert No. 11/2019) and *Augmentin* (Alert No. 9/2019) [1].

The authorized manufacturer, SmithKline Beecham Limited, confirmed that it had no connection with the falsified Augmentin batch. These alerts, which targeted Uganda and Kenya, represented the second WHO notification regarding counterfeit Augmentin in Africa — the first (2/2018) having been issued on March 2, 2018, for Cameroon. At that time, the manufacturer also denied producing the batch in question, and laboratory testing confirmed the absence of active ingredients and labeling inconsistencies [2].

Alert No. 11/2019 documented two falsified versions containing amoxicillin and clavulanic acid, detected in Haiti and sold as *Augmentin*, *Amoxicillin Potassium Clavulanate*, and *Bactoclav*. The alert was triggered by Haiti's Ministry of Health through the VigiCarib network of the Caribbean Public Health Agency. The products were identified in a pharmacy [3].

For the first product, NovoPharm Limited and TEVA Pharmaceuticals USA confirmed they had not manufactured it, and the label did not match any genuine series. In the *Bactoclav* case, Mylan stated it did not produce, subcontract, or distribute any such products. The French-language packaging displayed spelling and design inconsistencies. These falsified products were ineffective in bacterial infections and could contain unknown, potentially toxic excipients.

According to international data, between 1999 and 2002, antibiotics accounted for 28% of falsified medicines worldwide, and subsequently nearly half of reported cases occurred in developing countries. Key factors include limited access to quality medicines, unregulated parallel markets, weak regulatory oversight, and sometimes corruption in the supply chain.

The issue has worsened with increasing cross-border mobility. In some markets, antibiotics are available without prescription, encouraging self-medication and demand for cheaper alternatives, which exposes consumers to counterfeit products. The WHO and INTERPOL have documented massive seizures of falsified antibiotics in ports, airports, and local networks. Between 2014 and 2016, antibiotics represented 36% of all counterfeit medicines seized, with frequent involvement of beta-lactams and tetracyclines.

The clinical impact is twofold: (1) therapeutic failure, worsening infections, and potentially fatal complications; (2) sub-dosing or absence of the active substance promotes antimicrobial resistance — one of the greatest threats to global health. The WHO warns that this situation may reverse decades of progress in infectious disease control.

The most recent WHO alert on counterfeit antibiotics concerns *HEALMOXY 500 mg capsules* by Maxheal Pharmaceuticals Limited (India). In March 2025, the WHO issued an alert (Medical Product Alert No.

2/2025) about four falsified batches identified in Cameroon and the Central African Republic. Laboratory analyses revealed the absence of amoxicillin, indicating deliberate falsification of identity, composition, and origin, posing a serious public health risk. Label anomalies — such as date formats written in day/month/year order — inconsistent with authorized manufacturers' practices, were useful indicators for identifying falsifications [4].
In addition to official alerts, authorities have conducted seizures, recalls, and awareness campaigns. The lack of a unified definition and standardized global procedures has hindered coordination: the WHO defines "falsified" products as those that deliberately misrepresent identity or source, while the EMA introduces further distinctions, potentially creating ambiguity.

Alerts regarding counterfeit antibiotics must be seen as part of a persistent trend revealing weaknesses in supply and regulatory chains in certain regions. The solution requires an integrated approach: strengthening national frameworks, international cooperation, public education, and implementation of traceability/authentication technologies.

Operationally, anti-counterfeiting enforcement has relied on large-scale coordinated actions. INTERPOL conducts the annual *Operation PANGEA*, targeting online trade and illicit logistics networks. In 2023 (*PANGEA XVI*): 72 arrests, 325 new investigations, and over 1,300 websites shut down. In 2024–2025 (*PANGEA XVII*): 769 suspects, 123 dismantled criminal groups, and 50.4 million doses seized, valued at USD 65 million — actions that disrupted cross-border flows, closed unauthorized online pharmacies, and strengthened criminal prosecution [5].

On a systemic level, new verification and traceability tools have been introduced. Since 2010, Nigeria has used the *Mobile Authentication Service (MAS)*, featuring unique codes verified via SMS and NAFDAC guidelines [6]. Recently, Nigeria has begun transitioning to end-to-end traceability based on GS1 standards (2D DataMatrix and serialization), with pilot projects and dedicated regulations. Effectiveness depends on public awareness and adherence to standards across the entire supply chain. In practice, combining point-of-sale verification, serialization, targeted inspections, and cross-border cooperation has reduced counterfeit circulation, enabled early detection, and accelerated recalls.

The case-resolution pattern is relatively standardized: initial reporting (by patient or pharmacist), sample collection under post-marketing surveillance, laboratory testing, WHO/regional notification, official alert, recalls/seizures, followed by communication, extended inspections, and, where possible, criminal prosecution. In parallel: border controls, traceability verification, supplier audits, laboratory strengthening, and education of healthcare professionals and patients to recognize counterfeit signs.

In summary, WHO alerts from 2018–2019 regarding *Augmentin* (East Africa, Haiti) and the 2025 alert on *Healmoxy*(Central Africa) follow the same response logic: analytical confirmation of falsification, mobilization of regional/international regulatory channels, rapid recalls, and targeted communication to clinicians and patients.

Combined with enforcement operations (*PANGEA*) and traceability tools (MAS, GS1, serialization), these measures have reduced counterfeit circulation and improved early detection. However, the persistence of the phenomenon highlights the need for additional laboratory resources, alignment of terminology and reporting among jurisdictions, and continuous investment in traceability to protect the efficacy and safety of antibiotics.

2. Counterfeiting of Innovative Biological Medicines

The high cost, manufacturing complexity, and parenteral administration of biological medicines make them prime targets for counterfeiting. Counterfeit biologics may completely lack the active substance or contain dangerous excipients and contaminants, posing serious risks to patients with severe diseases such as cancer, autoimmune, or hematologic disorders. Common mechanisms include infiltration through parallel or online distribution channels and packaging that closely imitates the original but with subtle errors—leading to severe clinical consequences, ranging from lack of efficacy to serious or even fatal immune reactions. The following analysis groups the cases by therapeutic class.

2.1. Monoclonal Antibodies

Bevacizumab (Avastin)

Avastin, a monoclonal antibody used in colorectal, lung, and glioblastoma cancers, is a landmark case in the history of counterfeit biologics. In 2012, in the United States (California, Texas, Illinois), batches with no active substance were identified, originating from unauthorized distributors; similar cases were reported in Nigeria, Turkey (2011–2013), and Egypt (2018; *WHO Medical Product Alert No. 3/2017*) [7]. The counterfeits mimicked genuine vials but contained inert solutions. Consequences included loss of tumor control and patient deaths. Investigations by the FDA and the manufacturers (Genentech, Roche) revealed vulnerabilities in parallel distribution channels [8,9]. The response involved public alerts, enhanced traceability, and digital authentication technologies. This case remains a key reference for understanding the risks associated with innovative biologics [10,11].

Rituximab (MabThera/Rituxan)

Rituximab (anti-CD20), essential in the treatment of non-Hodgkin lymphoma, chronic lymphocytic leukemia (CLL), and autoimmune diseases, became a target due to its high therapeutic value and constant demand.

In 2012, Roche issued an alert regarding counterfeit MabThera batches in Germany, obtained through parallel imports, which contained only saline solution [12]. In August 2024, Nigeria's regulatory authority (NAFDAC) reported falsified packaging (500 mg/50 ml, batch N7458B07, exp. November 2022), initially distributed in Turkey in 2021 but later found in Nigeria. The labels showed automatic translations and discrepancies in the package inserts, and analyses confirmed the absence of the active ingredient [13]. In Sri Lanka (2024), criminal investigations targeted the distribution of counterfeit rituximab to public hospitals, involving vials with no active substance and possible complicity in the procurement chain [14]. In response, authorities implemented holographic labeling, unique serialization, digital codes, and collaboration with INTERPOL. The case has become a standard example of supply chain protection for biologic medicines [15,16].

3. Counterfeiting of Innovative Biological Medicines

The high cost, manufacturing complexity, and parenteral administration of biologics make them prime targets for counterfeiting. Fakes may completely lack active substance or contain dangerous excipients/contaminants, endangering patients with severe conditions (cancer, autoimmune, hematologic). Usual mechanisms: penetration via parallel or online channels, packaging nearly identical to originals but with subtle errors, and serious clinical consequences—from ineffectiveness to severe, sometimes fatal, immune reactions. The analysis below groups cases by therapeutic class.

3.1. Hematopoietic Growth Factors and Recombinant Proteins

Epoetin alfa (Eprex)

Epoetin alfa, used for anemia associated with renal failure, chemotherapy, HIV infection, or major surgery, has been a recurrent target. In Germany (1997–2002), episodes of pure red cell aplasia (PRCA) occurred in patients exposed to falsified/manipulated products; some vials came from parallel supply chains. Incorrect concentrations or degraded proteins induced neutralizing anti-erythropoietin antibodies; >200 PRCA cases worldwide often required transfusions or bone-marrow transplant. In Asia (China, Thailand 2005–2007), falsified vials were identified; among 62 samples from the authorized chain and 77 from outside it, counterfeit lots showed protein aggregates of 2.2–19.8% (vs <2%), increasing immunogenicity and PRCA risk [17,18]. Subsequently, EMA/FDA strengthened surveillance, PRCA reporting, serialization, and packaging measures (syringes with fixed needles) [19].

Recombinant Factors VIII and IX

Essential therapy in hemophilia A/B was affected by counterfeits (2005–2012), especially in Eastern Europe, Asia, and Africa. Packaging mimicked originals, but lots contained subtherapeutic doses, biologically inactive solutions, or degraded proteins [20]. Consequences: severe hemorrhage and deaths (reported in Nigeria, Sudan, etc.). Parallel distribution with serious clinical effects was identified in Russia and Ukraine. In response, the WFH, WHO, and national agencies intensified monitoring and reporting [21,22].

3.2. Hormones and Hormone Analogues

Insulin glargine (Lantus)

Insulin glargine (Sanofi) is widely used as a basal insulin. The first counterfeits were reported in China (2013): fake pens without insulin or with variable concentrations, associated with severe hyperglycemia, ketoacidosis, and repeated hospitalizations. In West Africa (2015–2018), WHO/INTERPOL checks seized counterfeit pens in Nigeria and Cameroon; packaging closely imitated originals but showed discrepancies in font/color/lot numbers. Labs identified sterile water or glucose solutions. Clinical cases included metabolic decompensation and DKA in Nigeria [23]. Similar situations were reported in Brazil and Mexico. Measures: security codes and serialization, QR verification apps, WHO/IDF education campaigns, cross-border cooperation (PANGEA). The problem remains current amid rising diabetes prevalence [24,25].

Somatropin (hGH)

Somatropin (recombinant growth hormone) is used in GH deficiency, Turner syndrome, pediatric CKD, Prader–Willi—and off-label (sport, "anti-aging") [26,27]. In East Asia (2000s), authorities seized vials/pens labeled hGH with no active substance or contaminated with bacterial proteins/endotoxins. Children experienced lack of growth progress, hypersensitivity, infections, and sepsis. In Europe (2014–2016), counterfeit pens circulated on the black market for athletes, causing severe local and systemic reactions. WADA notes that a substantial share of black-market hGH is counterfeit; up to 40–50% of online products fail to meet standards [28]. Impact: in children, irreversible compromise of growth; in adults, severe reactions and legal risks.

3.3. Recombinant Interferons

Interferon alfa and beta

Interferons alfa/beta—historically essential in viral hepatitis B/C (alpha) and multiple sclerosis (beta)—are fragile protein molecules, cold-chain dependent, and costly—conditions conducive to counterfeiting and iatrogenic degradation via parallel circuits. In Eastern Europe and Central Asia (early 2000s), vials labeled interferon alfa without active substance were reported; in Eastern Europe, MS relapses were later linked to interferon beta from unauthorized channels. Storage deviations (2–8 °C, protection from agitation/light) accelerate denaturation/aggregation, turning an apparently authentic product into an inactive one. Clinically: loss of efficacy,

frequent relapses, new MRI lesions, and increased immunogenicity (neutralizing antibodies). Investigations showed low potency in cell assays, aggregation above thresholds, and subtle labeling inconsistencies. Response: serialization, DataMatrix and electronic verification; surveillance focused on early clinical signs; policing digital markets and training professionals/patients on maintaining the cold chain [29,30].

3.4. Counterfeit Biological Vaccines

Rabies vaccine (China)

In 2010 in Guangxi, a counterfeit rabies-vaccine production/distribution chain reached clinics. The investigation followed the death of a 5-year-old (Laibin) who had received six injections of fake product; vials contained mainly water/saline. Over 1,656 people were vaccinated with fake doses between August–December 2009, most revaccinated by June 2010; eight defendants received prison sentences [31]. In 2018, the Changchun Changsheng scandal involved falsification of rabies-vaccine production data [32]. In 2019, WHO issued Medical Product Alert 1/2019 for counterfeit rabies vaccine in the Philippines; Sanofi stated the batches did not match its records [33]. Clinically, lack of immunization after rabies exposure—a disease with ~100% fatality after onset—requires urgent revaccination for exposed persons.

Meningococcal vaccine, Niger (1995 epidemic)

In 1995, amid an epidemic, Niger received 88,000 doses labeled as products of Pasteur Mérieux and SmithKline Beecham; it was later shown that some were counterfeit, lacking active antigen. An estimated ~60,000 people received inert doses, associated with thousands of deaths [34-36]. Suspicion arose from the absence of the expected post-campaign epidemiologic effect and from vial appearance; manufacturers confirmed counterfeiting [37]. Consequences: no immunization, increased mortality, erosion of trust. Lessons led to the 1997 creation of the International Coordinating Group (ICG) on Vaccine Provision (WHO, UNICEF, IFRC, MSF) for traceable supply in emergencies. In 2019, Niger again reported counterfeit versions of Mencevax ACWY (Alert 5/2019) [38].

3.5. Other Recent Innovative Biologics

Semaglutide (Ozempic)

Between 2023–2025, global demand increased the risk of counterfeiting. On June 19, 2024, WHO issued Medical Product Alert 2/2024 for three falsified lots in Brazil (10/2023), the UK (10/2023), and the USA (12/2023), which had infiltrated regulated supply chains. The manufacturer confirmed identity tampering (non-existent or reused lots). Since 2022, GSMS noted rising reports globally, publishing identification clues: atypical dose scales, poor labeling, spelling errors. Clinically, risks include inefficacy or severe reactions if substitutes/contaminants are present [39].

In the USA, on December 21–22, 2023, the FDA seized thousands of falsified Ozempic 1 mg units from the official supply chain (lot NAR0074, serial 430834149057) and banned their use/distribution. Update April 14, 2025: additional units seized (PAR0362, serials starting 51746517) and reports of non-serious adverse events. In the UK (October 18, 2023), the MHRA identified counterfeit pens at two wholesalers, withdrawn before reaching patients; on October 26, 2023, it warned about potentially fake pens and hospitalizations (including hypoglycemic shock/coma), later confirming cases with insulin instead of semaglutide. In Australia, the TGA reported on September 30, 2024, falsely labeled pens with a life-threatening event; on April 3, 2025, more pens were intercepted (lot MSPD916). In Romania, on August 4, 2025, ANMDM issued a preventive warning regarding a counterfeit product in Portugal (Ozempic 1 mg, lot NF63704; NovoFine Plus 32G 4 mm, lot NE61324 2), requesting prompt reporting [40].

Heparin (contaminated with OSCS)

In 2007–2008, injectable heparin lots contaminated with oversulfated chondroitin sulfate (OSCS) were identified, originating from raw materials processed in China. In the USA, anaphylactoid reactions, severe hypotension, and circulatory collapse were reported; 81 deaths and >785 serious events were investigated. OSCS constituted up to ~30% w/w in some lots, evading then-current compendial tests. Mechanism: activation of the plasma contact system with generation of kallikrein and bradykinin—explaining acute reactions, especially in dialysis patients. The supply chain showed major vulnerabilities (porcine starting material, upstream suppliers). Response: recalls in March 2008, followed by USP updates (2009, then 2012) with more specific methods (including NMR) and additional control requirements. The case redefined analytical standards and supplier qualification for tissue-derived products [41-43].

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4. Counterfeiting of PDE5 Inhibitors and Implications for Patient Safety

Sildenafil (Viagra)

Sildenafil is a constant target due to high demand, stigma, and online distribution. Independent audits have shown the scale: a Pfizer audit of 22 “top-search” websites found that 80% of units received as “authentic” were actually counterfeit; a parallel clinical study found 77% fakes among products purchased online as Viagra [44]. INTERPOL’s Operation PANGEA confirms the persistence of the phenomenon: in 2023, products for erectile dysfunction accounted for 22% of seizures; in 2025, PANGEA XVII mobilized 90 countries, making 769

arrests and seizing 50.4 million doses [45]. In the UK (June 25, 2025), the MHRA announced 7.7 million illegal doses seized. Laboratory analyses showed wide variability in content and characteristic impurities, including undeclared PDE5 analogues [46]. A particular risk is posed by "natural" supplements deliberately adulterated with sildenafil/tadalafil (the FDA maintains alert lists), with potential for dangerous interactions (e.g., with nitrates), severe hypotension, syncope, or cardiac events. Recent examples: seizures in Canada (May 2025) and repeated MHRA actions in the UK [47].

Tadalafil (Cialis)

Tadalafil is frequently counterfeited both as "Cialis" and in "natural" products containing undeclared tadalafil. In the UK, as early as 2004, counterfeit lots of Cialis 20 mg were identified that had entered the regulated supply chain [48-50].

Vardenafil (Levitra)

Vardenafil (Levitra) has followed the same pattern. A study (Japan, April 2015) purchased 28 online samples labeled "Vardenafil 20 mg" from 15 websites and applied screening (NIR/Raman, quantitative analyses). Results: 60% fakes; all authentic products passed tests, while some counterfeits contained sildenafil or tadalafil instead of vardenafil, with high variability in spectra and content. Portable Raman/NIR devices proved useful for rapid triage before HPLC-MS confirmation [51-54].

5. Conclusion

The case analyses show that counterfeit medicines pose a major, persistent global public-health threat, affecting both traditional medicines (e.g., antibiotics) and innovative biologics or erectile-dysfunction drugs. For antibiotics, counterfeiting has led not only to therapeutic failures and severe individual complications, but also to a global-impact phenomenon—rising antimicrobial resistance. WHO alerts and INTERPOL's PANGEA operations indicate a steady increase in seized counterfeits and diversification of sources across Africa, Asia, and Latin America. Innovative biologics—monoclonal antibodies, growth factors, recombinant hormones, interferons, and vaccines—are targeted due to high cost and demand; counterfeits have dramatic consequences: lack of efficacy, severe immune reactions, relapses, and deaths. Landmark cases (Avastin, MabThera, Eprex, Lantus, semaglutide, and contaminated heparin) have driven stronger traceability, serialization, analytical control, and cold-chain standards.

For PDE5 inhibitors (sildenafil, tadalafil, vardenafil), commercial incentives and social stigma fuel illegal online trade; counterfeits often contain variable doses or toxic substances, posing major cardiovascular risks.

All cases point to the same conclusions:

- Counterfeiting is a transnational, complex, and dynamic phenomenon, adapting to the evolving pharmaceutical market.
- Effective response requires international cooperation (WHO, INTERPOL, EMA, national authorities), modern traceability/authentication tools (MAS, DataMatrix, GS1, QR codes), public education, and professional responsibility across the supply chain.
- Ongoing surveillance, laboratory capacity-building, and harmonized terminology/legal reporting across jurisdictions are essential to reduce risks.
- Ultimately, combating counterfeit medicines goes beyond seizures and alerts; it is a strategic component of global medicines safety and of safeguarding patient trust in health systems.

References

1. *https://www.who.int/news/item/20-08-2019-medical-product-alert-n-9-2019-(english-version)*
2. *https://www.who.int/news/item/02-03-2018-medical-product-alert-n-2-2018--falsified--augmentin*
3. *https://www.who.int/news/item/16-10-2019-medical-product-alert-n-11-2019-(english-version)*
4. *https://www.who.int/news/item/23-04-2025-medical-product-alert-n-2-2025--falsified-healmoxy-(amoxicillin)-capsules-500mg*
5. *https://www.interpol.int/News-and-Events/News/2025/Record-769-arrests-and-USD-65-million-in-illicit-pharmaceuticals-seized-in-global-bust*
6. *https://nafdac.gov.ng/category/recalls-and-alerts/*

7. *https://www.who.int/news/item/18-08-2017-medical-product-alert-n-3-2017--falsified-avastin-and-sutent*
8. *https://www.gene.com/download/pdf/avastin_DHCP_022012.pdf*
9. *https://globalinitiative.net/wp-content/uploads/2017/12/IRACM-Counterfeit-Medicines-and-Criminal-Organizations-Oct-2013.pdf*
10. *T. Almuzaini, I. Choonara, H. Sammons, Substandard and counterfeit medicines: a systematic review of the literature, BMJ Open, aug 2013, doi: 10.1136/bmjopen-2013-002923*
11. *Bea Perks, Pharmaceutical fakes: a dangerous pandemic, mai 2015, https://pharmaceutical-journal.com/article/feature/pharmaceutical-fakes-a-dangerous-pandemic*
12. *Roche, Roche warns once again of counterfeits of its biologi drugs, Securing Industry, 2012, https://www.securingindustry.com/pharmaceuticals/roche-warns-once-again-of-counterfeits-of-its-biologic-drugs/s40/a2132/*
13. *NAFDAC, Public Alert no 037/2024. Alert on confirmed counterfeit of MabThera 500mg/50ml in Nigeria, https://nafdac.gov.ng/public-alert-no-037-2024-alert-on-confirmed-counterfeit-of-mabthera-500mg-50ml-in-nigeria/*
14. *https://lankanewsweb.net/archives/53904/counterfeit-cancer-drugs-scandal-unveiled-patients-given-fake-rituximab/*
15. *Robert Cockburn, Paul Newton, E Kyerematen Agyarko, Dora Akunyili, Nicholas J White, The Global of Counterfeit Drugs: Why Industry and Governments Must Communicate the Dangers, PLosMed, 2005 mar 14; 2(4). https://doi.org/10.1371/jpurnal.pmed.0020100*
16. *Aonghus J Feeney, Jeffery A Goad, Gerard T Flaherty, Global perspective of the risks of falsified and counterfeit medicins: A critical review of the literature, Travel Medicine and Infectious Disease, volume 61, September-octomber 2024, 102758, https://doi.org/10.1016/j.tmaid.2024.102758*
17. *Fotis Fotiou, Suresh Aravind, Wang Ping, Osot Nerapuse, Impact of illegal trade on the quality of epoetin alfa in Thailand, Clinical Therapeutics, feb. 2009, 31(2):336-46, https://doi.org/10.1016/J.CLINTHERA.2009.02.014*
18. *Alexandre Marchand, Laurent Martin, Jean Antoine Martin, Magnus Ericsson, Michel Audran, The case of the EPO poisoned syringe, Drug Testing and Analysis. Special issue case report, 30 dec 2019, https://doi.org/10.1002/dta.2757*
19. *Bryan A Ling, Timothy Mackey, Biosimilars and patient safety risk: promoting policy protections in the health delivery system, Reviews in Health Care 2012, 3(2):71-74*
20. *Pfizer, Insinghts into Illegal and Counterfeit Drugs, https://www.pfizer.com/news/articles/fake_drugs_101_facts_on_illegal_counterfeit_drugs*
21. *WHO: World Federation of Hemophilia Report on the Annual Global Survey, 2022, https://www1.wfh.org/publications/files/pdf-2399.pdf*
22. *Newsroom: ISTH News, Critical Juncture in Hemophilia Treatment: Global Organizations Issue Urgent Call to Action, may 2025, https://www.eahad.org/critical-juncture-in-hemophilia-treatment-global-organizations-issue-urgent-call-to-action/*
23. *https://www.interpol.int/en/Crimes/Pharmaceutical-crime*

24. *Kartika Saraswati, Chanvilay Sichanh, Paul Newton, Celine Caillet, Quality of medical products for diabetes management: a systematic review¸BMJ Glob Health, 2019, sep 2024, 4(5), doi:10.1136/bmjgh-2019-001636*
25. *https://www.fda.gov/drugs/buying-using-medicine-safely/counterfeit-medicine*
26. *https://www.accessdata.fda.gov/cms_ia/importalert_204.html*
27. *https://www.fda.gov/consumers/health-fraud-scams/health-fraud-product-database*
28. *https://pmc.ncbi.nlm.nih.gov/articles/PMC6116101/*
29. *Per Soelberg Sorensen, Neutralizind Antibodies Against Interferon Beta, Ther Adv Neurol Disord, sept 2008, 1(2):62-78*
30. *WHO. Substandard and falsified medical products: Overview and WHO response, https://www.who.int/news-room/fact-sheets/detail/substandard-and-falsified-medical-products*
31. *https://www.scmp.com/article/726012/1600-get-fake-rabies-vaccine*
32. *https://www.bbc.com/news/world-asia-china-45886388*
33. *https://www.who.int/news/item/01-02-2019-medical-product-alert-n-1-2019*
34. *Amir Attaran, Roger Bate, Megan Kendall, Why and how to make an international crime of medicine counterfeiting, Journal of International Criminal Justice, volume 9, issue 2, may 2011, pag 325-354, https://doi.org/10.1093/jicj/mqr005*
35. *Idris Mohamed, Garba Iliyasu, Abdulrazaq Garba Habib, Emergence and control of epidemic meningococcal meningitis in sub Saharan Africa, Pathlog Glob Health, 2017, 111(1):1-6, https://pmc.ncbi.nlm.nih.gov/articles/PMC5375607/*
36. *https://cdn.pfizer.com/pfizercom/products/DevelopingWorld.pdf*
37. *Pierre Saliou, Quentin Duteil, Stanley A Plotkin, Marc Gentilini, The scourge of vaccine falsification, Vaccine, feb 2022, 40(14):2126-2128, doi:10.1016/j.vaccine.2022.01.063*
38. *https://www.who.int/news/item/28-01-2020-medical-product-alert-n-5-2019-(english-version)*
39. *https://www.who.int/news/item/19-06-2024-medical-product-alert-n-2-2024--falsified-ozempic-(semaglutide)*
40. *https://www.ozempic.com/content/dam/novomedlink/semaglutide/04-14-25-company-statement.pdf*
41. *ANMDM, Comunicare directă către profesioniștii din domeniul sănătății, Ozemoic, 23 feb 2023*
42. *Tina S Morris, USP Further Strengthens Quality Standards for Heparin, Pharmaceutica Technology, 2012, vol 36, issue 9, https://www.pharmtech.com/view/usp-further-strengthens-quality-standards-heparin-0*
43. *Nicola Volpi, Francesca Maccari, Jiraporn Suwan, Robert J Linhardt, Electrophoresis for the analysis of heparin purity and quality, Electrophoresis, 2012, 33(11):1531-1537*
44. *Tanja Beyer, Magnus Matz, Daniela Brinz, Oliver Radler, Brnhard Wolf, Jochen Norwig, Knut Baumann, Susanne Alban, Ulrike Holzgrabe, Composition of OSCS contaminated heparin occurring in 2008 in bathes on the German market, Eur J Pharm Sci, 2010, 40(4):297-304*
45. *https://www.interpol.int/News-and-Events/News/2025/Record-769-arrests-and-USD-65-million-in-illicit-pharmaceuticals-seized-in-global-bust*

46. *Claudia Rosito Jung, Rafael S Ortiz, Renata Limberger, Paulo Mayorga, A new methodology for detection of counterfeit Viagra and Cialis tablets by image processing and statistical analysis, Forensinc Science International, september 2021, 216(1-3):92-6, doi:10.106/j.forsciint.2011.09.002*
47. *FDA Notification: Secret miracle honey extra strength may be harmful due to hidden drug ingredients, iulie 2025, https://www.fda.gov/drugs/medication-health-fraud/secret-miracle-honey-extra-strength-may-be-harmful-due-hidden-drug-ingredients*
48. *Rafael Scorsatto Ortiz, Kristiane de Cassia Mariotti, Renata Pereira Limberger, Paulo Mayorga, Physical profile of counterfeit tablets Viagra and Cialis, Brazilian Journal of Pharmaceutical Sciences vol. 48, no 3, jul/sep 2012*
49. *RIVM, Recent developments in counterfeits and imitations of Viagra, Cialis and Levitra. A 2005-2006 update, https://www.rivm.nl/bibliotheek/rapporten/370030001.pdf*
50. *FDA Notification: Black horse miracle honey for men may be harmful due to hidden drug ingredients, aug 2025, https://www.fda.gov/drugs/medication-health-fraud/black-horse-miracle-honey-men-may-be-harmful-due-hidden-drug-ingredients*
51. *Shu Zhu, Naoko Yoshida, Kazuko Kimura, Ryo Matsushita, Hirohito Tsuboi, Falsified vardenafil tablets available online, JPharm Biomed Anal, 2020, 5:177:112872, PMID: 31525574, DOI:10.1016/j.jpba.2019.112872 https://pubmed.ncbi.nlm.nih.gov/31525574/*
52. *Sanada T, Yoshida N, Kimura K, Tsuboi H, Discrimination of falsified erectile dysfunction medicines by use of an ultra-compact Raman scattering spectrometer, Pharmacy (Basel), 2021, 9(1):3, https://pmc.ncbi.nlm.nih.gov/articles/PMC7839056/*
53. *Sanada T, Yoshida N, Kimura K, Tsuboi H, Detection method of falsified medicines by using a low cost Raman scattering spectrometer combined with soft independent modeling of class analogy and partial least squares discriminant analysis, Biol Pharm Bull, 2021, 44(5):691-700, doi:10.1248/bpb.b20-01041, https://pubmed.ncbi.nlm.nih.gov/33952825/*
54. *Chunzheng Li, Xinzheng Wang, Shengming Wu, Junqing Zhao, Junjian Fang, Hui Li, Yingjie Zhu, Kang Zhang, Jing Peng, Jie Mao, Weihua Li, Kun He, Na Wang, Fangting Dong, Separation and structural elucidation of a novel vardenafil analogue as an adulterant in a natural health care dietary supplement, Heliyon, aprilie 2023, 9(4):e15418, doi:10.1016/j.heliyon.2023, https://pubmed.ncbi.nlm.nih.gov/37128339/*

ANTHOLOGY OF TRADITIONAL ROMANIAN REMEDIES USED UP TO THE EARLY 20TH CENTURY

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DOI: https://doi.org/10.66793/titum19proceeding14

Abstract

Traditional Romanian medicine, preserved and transmitted through empirical observation, trial-and-error experimentation, and practical experience, represents a valuable source of historical therapeutic knowledge. This study presents a systematic analysis of 295 remedies documented in Dr. Vasile Voiculescu's "Toate leacurile la îndemână" (1935), highlighting their origin (plant, animal, mineral, microbiological, synthetic, or other) and type of therapeutic action (etiological, symptomatic, adjuvant, or miscellaneous). Plant-derived remedies predominated, and symptomatic applications were most frequent, reflecting the empirical priorities of past medical practice. The remedies targeted over 1,045 diseases, demonstrating the diversity and multifunctionality of traditional treatments. Toxicological and precautionary considerations were also recorded for certain substances. This anthology illustrates how traditional remedies have informed modern pharmacology, with many historical preparations serving as precursors for contemporary medicines. The systematic documentation and analysis of these remedies offer insights for ethnopharmacology, drug discovery, and the preservation of cultural medical heritage.

Keywords: traditional medicine, Romanian remedies, ethnopharmacology, historical therapeutics, phytotherapy.

INTRODUCTION

Medicine is based on the prevention, diagnosis, treatment, and rehabilitation of patients, aiming to maintain and restore the health of the population, understood as a state of physical, mental, intellectual, and social well-being. From this derives the social importance of medical personnel. Treatment, with all its stages and subdivisions, may be pharmacological (using medicines from plant, animal, microbial, mineral, or synthetic chemical origin) and/or non-pharmacological (employing various physical, chemical, biological, psychological, or surgical means).

Modern medicine has made significant progress, particularly in public health, through the practical application of individual and collective hygiene measures, the promotion of a healthy lifestyle, asepsis and antisepsis, surgical techniques, antibiotic therapy, and vaccination, using efficient methods and technologies. These advances have led to a doubling of human life expectancy, especially in wealthy countries, but they have also increased the complexity of medical practice and its direct and indirect costs. However, with the notable exception of infectious and contagious diseases, medical statistics have remained approximately at the same parameters regarding morbidity and recovery, even though costs have risen dramatically and sometimes become prohibitive. This situation has been influenced by health insurance systems, which have made the medical industry more profitable, as well as by the transformation of the pharmaceutical field from a health-oriented domain into an industrial one, alongside the industries of medical devices and equipment.

To gain an overall perspective on current therapeutic progress, it is necessary to compare it with traditional therapeutics from a century ago, a time before the discovery of antibiotics. For example, the great

microbiologist and immunologist Prof. Dr. Ioan Cantacuzino himself died of bacterial pneumonia in 1934, shortly before the rising of the antibiotic era. - microbiological parasitism predominated, involving viruses, bacteria, fungi, protozoa, and animals that cause infections and infestations, often manifesting as severe, disabling, or even fatal diseases. Some of these can be transmissible, causing outbreaks, epidemics, or pandemics, and in animals, epizootics and panzootics, posing a serious threat to public health. Therefore, the emergence and introduction into medical practice of antimicrobial chemotherapeutic agents (antibacterial - bactericidal and bacteriostatic antibiotics - antiviral, antifungal, and antiparasitic medicines) represented a major achievement of medicine, in collaboration with the pharmaceutical industry, which supplies the necessary pharmacological therapies.

The current level of therapy is well known and generally accessible, at least conceptually, but it must be compared with that of a century ago, particularly regarding population treatment, public health, and self-therapy, which has been - and remains - the foundation of prevention and treatment of mild, asymptomatic, oligosymptomatic, and transient diseases. These are the most common conditions, both at the individual and collective level. More severe diseases, with moderate or serious symptoms, require medical consultation, clinical and paraclinical examination, bed rest or hospitalization, and sometimes specialized intensive care, which nevertheless does not always guarantee recovery. Ultimately, every living being, healthy or ill, will die - but what truly matters are the conditions and the age at which this occurs.

Traditional medicine has played a central role in health care across cultures for centuries, drawing upon local knowledge, natural resources, and community practices to maintain well-being and treat illnesses. According to WHO, around 40 % of today's pharmaceutical compounds stem from nature and traditional medicinal practices, underscoring how deeply modern medicine is rooted in these earlier systems. In the context of this study, the examination of Romanian traditional remedies within this global framework highlights the relevance of integrating historical empirical data with current pharmacological, toxicological and standardisation approaches to develop safe, effective therapeutic solutions [1, 2, 3].

MATERIAL AND METHODS

To assess the therapeutic practices used a century ago among the Romanian population, we relied on a medical synthesis compiled by a renowned Romanian physician, writer, poet, and scientist. This work was published with the aim of educating and disseminating basic medical knowledge to the general public. We analyzed the content of *Dr. Vasile Voiculescu – "Toate leacurile la îndemână"* (*All Remedies at Hand*), part of the *Cartea Satului* Collection, Royal Cultural Foundation "Prince Carol", 2nd Edition, Bucharest, 1935, pp. 39–336, comprising a total of 297 traditional medical remedies [4]. We consider the efficiency to be very good when is higher than 75%.

The medical information was systematized in an alphabetically organized summary table, according to the predominant type of pharmacological action (etiological, symptomatic, adjuvant, or unspecified) and according to the source (plant, animal, microbial, mineral, synthetic chemical, or other). For each entry, the number of diseases treated, precautions, and relevant observations were recorded. The table begins with the letter *A* ("aburi" – vapors) and ends with *Z* ("zer" – whey), preserving the original terminology while including concise updated explanations.

This table can be subdivided and analyzed according to various criteria of interest—for therapists, pharmacists, or historians—as well as for the general public interested in traditional medicine.

RESULTS

Data on traditional Romanian remedies (e.g.) used until the early twentieth century are summarized in Table I

Table I. Traditional therapeutic remedies recommended by Dr. Vasile Voiculescu (1935), indicating their type of therapeutic action and source of origin (First 50)

No.	Popular Name	Scientific Name	Therapeutic Action	Source	Diseases Treated	Notes
1	Steam inhalation		Symptomatic	Water vapor	4	
2	Boric acid	Acidum boricum	Etiologic	Mineral	7	
3	Phenic acid	Fenolum	Etiologic	Chemical	2	Very toxic

4	Lactic acid	Acidum lacticum	Etiologic	Chemical	4	Very toxic
5	Salicylic acid	Acidum salicilicum	Etiologic	Chemical	2	Toxic
6	The air		Symptomatic	Air	10	
7	Bilberry	Vaccinum vitis idea	Symptomatic	Plant	6	
8	Bees	Apis mellifera	Adjuvant	Animal	3	
9	Eye ointment	Hydrargiri unuentum	Adjuvant	Chemical	3	
10	Purple ointment	Unguentum cum cupri salibus	Etiologic	Chemical	4	
11	Dough		Symptomatic	Plant	2	
12	Hazelnut	Coryllus avellana	Symptomatic	Plant	2	
13	Bitterness	Polygala vulgaris	Adjuvant	Plant	3	
14	Ammonia	Amonii hydroxydum	Etiologic	Chemical	5	Toxic
15	Anise	Pimpinella anisum	Symptomatic	Plant	5	
16	Angelica	Angelica archangelica	Symptomatic	Plant	6	
17	Artichoke	Cynara scolymus	Adjuvant	Plant	5	
18	Antipyrine	Phensonum	Symptomatic	Chemical	10	
19	Water		Etiologic	Water	5	
20	Breza water	Aqua mineralis	Symptomatic	Mineral	1	
21	Căciulata water	Aqua mineralis	Symptomatic	Mineral	1	
22	Flower water	Aurantii aqua	Symptomatic	Plant	1	
23	Mouthwash		Symptomatic	Chemical	3	
24	Hydrogen peroxide	Solutio Peroxydi diluta 3%	Etiologic	Chemical	5	
25	Lead water	Solutio plumbi acetatis basica	Etiologic	Chemical	5	
26	Pepper	Capsicum anuum	Symptomatic	Plant	2	Irritant
27	Alder	Alnus glutinosa	Symptomatic	Plant	3	
28	Arnica	Arnica montana	Symptomatic	Plant	2	Poisonous if ingested
29	Parsley variety	Petroselinum crispum	Symptomatic	Plant	2	
30	Aspirin	Acidum acetilsalicilicum	Symptomatic	Chemical	6	
31	Copaiba Balm	Balsamum Copaibae	Adjuvant	Plant	1	A.R.. diarrhea
32	Meadowsweet	Spiraea ulmaria	Symptomatic	Plant	9	
33	Laburnum	Laburnum anagyroides	Adjuvant	Plant	1	Toxic
34	Benzonaphthol	Benzoate of naphthol	Etiologic	Chemical	2	
35	Baking Soda	Sodium bicarbonate	Symptomatic	Chemical	2	
36	Bismuth	Bismuth subnitrate	Symptomatic	Chemical	3	
37	Fava Bean	Vicia faba	Adjuvant	Plant	1	Only alleviates
38	Borax	Sodium borate	Etiologic	Chemical	4	
39	Dwarf Elder	Sambucus ebulus	Symptomatic	Plant	4	
40	Hedge Mustard	Sisymbrium officinale	Symptomatic	Plant	2	
41	Autumn Crocus	Colchicum autumnale	Symptomatic	Plant	2	Toxic
42	Sodium/Potassium Bromide	Sodium/Potasium bromidum	Symptomatic	Chemical	2	
43	Peony	Paeonia	Symptomatic	Plant	5	
44	Basil	Ocimum basilicum	Symptomatic	Plant	2	
45	Coffee	Coffea arabica	Symptomatic	Plant	5	

46	Tansy	Tanacetum balsamita	Symptomatic	Plant	1
47	Camphor	Extracted from Cinnamomum camphora	Symptomatic	Plant	4
48	Honeysuckle	Lonicera caprifolium	Symptomatic	Plant	3
49	Raw Meat	—	Symptomatic	Animal	2
50	Potato	Solanum tuberosum	Symptomatic	Plant	2

DISCUSSIONS

By analyzing the alphabetical table of traditional remedies recommended to the population in the cited work, it can be observed that, according to their origin, 164 remedies are of plant origin (56%), 32 of animal origin (11%), 11 of mineral origin (4%), 61 of synthetic chemical origin (21%), 5 of microbiological origin (2%), and 18 of various other origins (6%), resulting in a total of 297 remedies (100%). Remedies of plant origin clearly predominate - more than all the others combined. It is no coincidence that the symbol of pharmacy is the green cross, representing the color of chlorophyll, in contrast to the symbol of medicine, the red cross, associated with hemoglobin.

From a pharmacological perspective, the therapeutic effect is etiological for 23 remedies (8%), symptomatic for 180 (61%), adjuvant for 97 (33%), and classified as miscellaneous for 15 (5%). A total of 315 therapeutic actions were recorded, since some remedies fall under more than one pharmacological category, thus totaling 107%. Remedies with symptomatic effects predominate, outnumbering all others combined, while etiological ones account for only 7%.

The total number of diseases treated with these remedies exceeds 1,045, with an average of about four diseases per remedy. The remedies with the widest therapeutic uses include Calendula (16 uses), milk (15), massage (14), honey (12), iodine tincture (11), and antipyrine, horseradish, and fresh air (10 each).

Some remedies are listed with miscellaneous uses that cannot be precisely quantified, such as sera and vaccines (Cantacuzino Institute was already operating at that time!), syringes, suppositories, vitamins, cotton, vegetable soup, and pork lard, among others.

Toxicity warnings are recorded for sixteen remedies, including phenic acid (liquefied phenol), lactic acid, ammonia, arnica, yellow acacia, colchicum, Aquilegia, Digitalis, ferns, Datura, laurel, garden poppy, henbane, mercury (salts), and opium, as well as other contraindications (age, comorbidi - often as self-medication or empirical treatment - was quantitatively and qualitatively diverse, yet their therapeutic efficacy was limited. Most of these remedies could be prepared in pharmacies or at home by the patient or caregiver, without standardization. At the time these data were collected, the chemical and pharmaceutical industries were already fairly developed, but in popular medical practice, traditional phytotherapy - inherited from ancient times - predominated.

Nowadays, all medicines are carefully tested and must meet strict quality standards. Industrial, galenic, and magistral preparations are produced in specialized institutions, while some, such as phytopharmaceutical preparations, can still be prepared at home from standardized “medicinal teas” or from wild or cultivated plants collected individually. Today, more plant extracts used in phytotherapy are standardized and produced industrially.

It can be observed that traditional medicine has been preserved through empirical filtering, trial-and-error experimentation, and careful observation, continuing up to the present day. These remedies were later subjected to laboratory testing and evaluation from botanical, physicochemical, microbiological, toxicological, and pharmacological perspectives, leading to the selection, standardization, and quantification of pharmacologically active concentrations to achieve the desired therapeutic effect with minimal adverse effects. Many modern medicines originate from these traditional remedies, now formulated in modern pharmaceutical forms [5].

When comparing the therapeutic approaches of a century ago with modern therapeutics - both pharmacological and non-pharmacological - the enormous progress becomes evident, particularly in synthetic chemical medicines and pharmaceutical biotechnology.

CONCLUSIONS

A century ago, traditional medicine offered a wide range of remedies from various origins, but their therapeutic efficacy was relatively limited.

The advances of modern medicine have been made possible in part thanks to the pharmaceutical industry, but they are also grounded in the medical-pharmaceutical knowledge preserved by the population and subsequently validated scientifically for practical use.

In just one century, therapeutic approaches have progressed both quantitatively and qualitatively, contributing to the maintenance of public health and the recovery of patients. Exploring and utilizing traditional remedies based on pharmacologically active principles derived from the living world - microorganisms, plants, fungi, and animals - represents an optimal pathway for developing new, effective, and safe medicines, deserving the attention of researchers in the medical and pharmaceutical fields.

Bibliography

1. *WHO. Traditional medicine has a long history of contributing to conventional medicine and continues to hold promise. World Health Organization, 2023*
2. *Yuan, H.; Ma, Q.; Ye, L.; Piao, G. The Traditional Medicine and Modern Medicine from Natural Products. Molecules* ***2016****, 21(5), 559. https://doi.org/10.3390/molecules21050559*
3. *Surve, M.V.; Shyamsundar, A.S.; Jatale, A.P.; Deshmukh, S.P. A review on current scenarios of pharmaceutical and herbal medicine and future prospects. GSC Biological & Pharmaceutical Sciences* ***2024****, 27(02), 49–59. https://doi.org/10.30574/gscbps.2024.27.2.0168*
4. *Vasile Voiculescu – Toate leacurile la îndemână, colecția Cartea satului, Fundaţia Culturală Regală ,,Principele Carol", Ediția a-II-a, Bucureşti, 1935, pp 39-336*
5. *Dias, D.A.; Urban, S.; Roessner, U. A Historical Overview of Natural Products in Drug Discovery. Metabolites* ***2012****, 2(2), 303–336. https://doi.org/10.3390/metabo202030*

OPTIMIZING DRUG BIOAVAILABILITY USING SUPERCRITICAL FLUIDS: DEVELOPMENT OF AN ANALYTICAL DISSOLUTION TEST METHOD FOR RIVAROXABAN

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DOI: https://doi.org/10.66793/titum19proceeding15

Abstract:

The paper summarizes the dissertation "Optimization of Drug Bioavailability through the Use of Supercritical Fluids. Development of an Analytical Dissolution Testing Method for Rivaroxaban", with the following objectives:
(i) to present technologies based on supercritical fluids (particularly supercritical CO_2) for improving the bioavailability of poorly soluble substances, and (ii) to develop and validate an analytically supported dissolution method using HPLC for rivaroxaban.
Dissolution tests were performed using the USP Apparatus 2 (paddle) at 75 rpm, 37 ± 0.5 °C, in acetate buffer pH 4.5 (without SLS), with sampling at 10–60 minutes. Quantification was carried out by HPLC (Agilent 1260 Infinity II, λ = 249 nm), with a retention time of approximately 4.15 minutes and a linear calibration range of 0.5–10 µg/mL. The dissolution profiles (n = 6) showed a rapid initial increase (≈27% at 10 minutes), followed by a plateau at ≈27% at 60 minutes, with low variability, confirming the discriminatory and reproducible character of the method under restrictive conditions. The results support the use of the dissolution method as an analytical framework for evaluating future prototypes obtained through supercritical fluid technologies, with potential to enhance dissolution rate and systemic exposure for BCS Class II drugs.

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Keywords: *supercritical fluids, $scCO_2$, rivaroxaban, HPLC, dissolution test, bioavailability, BCS Class II, SAS, RESS*

1. INTRODUCTION

Bioavailability determines the rate and extent to which an active pharmaceutical ingredient reaches systemic circulation, directly shaping therapeutic response and dose selection. The dissertation positions rivaroxaban as a Biopharmaceutics Classification System (BCS) Class II drug—high permeability but low aqueous solubility—where dissolution is the limiting step for oral absorption. In parallel, supercritical fluids (SCFs), particularly supercritical CO_2 ($scCO_2$), are presented as green, tunable media enabling particle size reduction, polymorph control, encapsulation and controlled release, with demonstrated benefits for poorly soluble drugs in the literature cited within the dissertation.

The work combines a comprehensive theoretical overview of SCF-based processes (e.g., RESS and SAS) with an experimental, HPLC-supported dissolution method for rivaroxaban under restrictive conditions (acetate buffer pH 4.5 without surfactant). This analytical platform is intended to be the baseline for future SCF-derived prototypes (nanocrystals, amorphous solid dispersions) and for in vitro–in vivo linkages.

The article distills two complementary objectives reflected in the dissertation: (1) to outline how supercritical-fluid technologies can optimize the bioavailability of poorly water-soluble drugs, and (2) to develop and validate a discriminatory dissolution method for rivaroxaban supported by HPLC quantitation. The study design includes a theoretical synthesis of SCF methodologies, plus a practical dissolution/HPLC workflow on commercial rivaroxaban tablets (20 mg).

2. MATERIALS AND METHODS

Dissolution testing employed USP Apparatus 2 (paddles) at 75 rpm and 37 ± 0.5 °C in acetate buffer pH 4.5, intentionally formulated without sodium lauryl sulfate (SLS) to emphasize dissolution-limited behavior under restrictive, biorelevant conditions. Six vessels were sampled at 10, 20, 30, 40, 50 and 60 minutes. Samples were prepared and analyzed by HPLC.
HPLC conditions: Agilent 1260 Infinity II equipped with UV detection; mobile phase consisting of acetonitrile and acetate buffer pH 4.5 adjusted for peak symmetry; monitoring at $\lambda = 249$ nm; retention time for rivaroxaban of ~4.15 minutes. Calibration covered 0.5–10 µg/mL with linear regression described by Area = 14320.98 × C + 1268.32. Limits were LOD = 0.00055 µg/mL and LOQ = 0.00167 µg/mL.

3. RESULTS AND DISCUSSION

Dissolution profiles across six vessels revealed a rapid initial rise followed by stabilization at ~27% dissolved by 60 minutes. The low standard deviations across time points indicate good lot homogeneity and method reproducibility under restrictive conditions.

Table. 1 Dissolution of rivaroxaban 20 mg in acetate buffer pH 4.5 (n = 6)

Time (min)	V1 (%)	V2 (%)	V3 (%)	V4 (%)	V5 (%)	V6 (%)	Mean (%)	SD
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	21.75	27.45	28.13	29.90	29.90	28.65	27.63	2.774977
20	24.25	25.21	26.99	26.64	27.80	28.35	26.54	1.419460
30	24.23	25.79	28.04	25.70	28.67	26.76	26.53167	1.496612
40	25.74	23.88	28.18	30.55	26.40	27.37	27.02	2.074102
50	23.95	24.68	26.27	26.82	26.86	26.92	25.91667	1.171504
60	27.39	26.62	27.45	27.55	25.98	28.06	27.175	0.680900

HPLC quantitation confirmed a stable retention time (~4.15 min) and linear detector response. The regression (Area = 14320.98 × C + 1268.32) provided excellent linearity ($R \approx 0.9977$; $R^2 \approx 0.9954$).

The method sensitivity was supported by very low LOD (0.00055 µg/mL) and LOQ (0.00167 µg/mL).

The restrictive, surfactant-free medium intentionally exposed the dissolution-limited behavior typical of BCS Class II drugs such as rivaroxaban. The plateau around ~27% dissolved after 60 minutes, coupled with low inter-vessel variability, indicates a discriminatory yet reproducible method suitable for lot-to-lot comparison and for assessing formulation changes. In the dissertation, SCF processes (RESS, SAS) are proposed to generate fine or amorphous particles, potentially increasing the dissolution rate and exposure (AUC, Cmax) for rivaroxaban once prototypes are available.

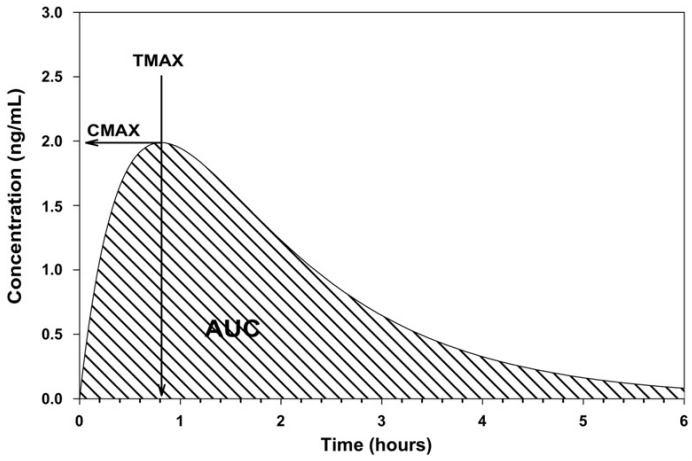

Figure 1. Representative illustration related to SCF processing, HPLC analytics, or dissolution workflow.

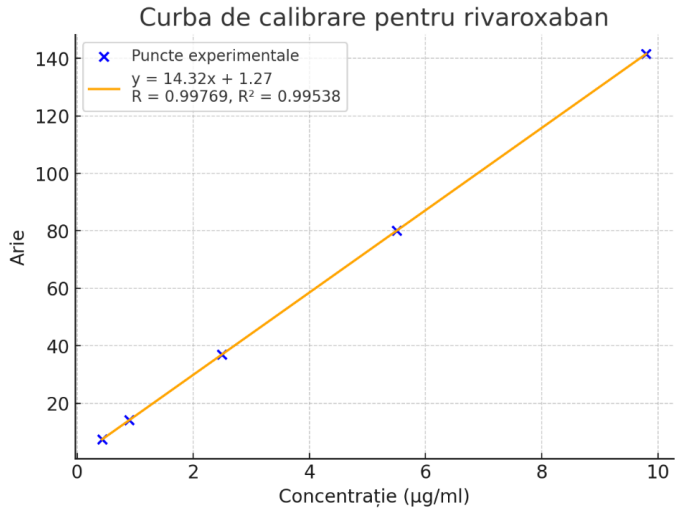

Figure 2. Representative illustration related to SCF processing, HPLC analytics, or dissolution workflow.

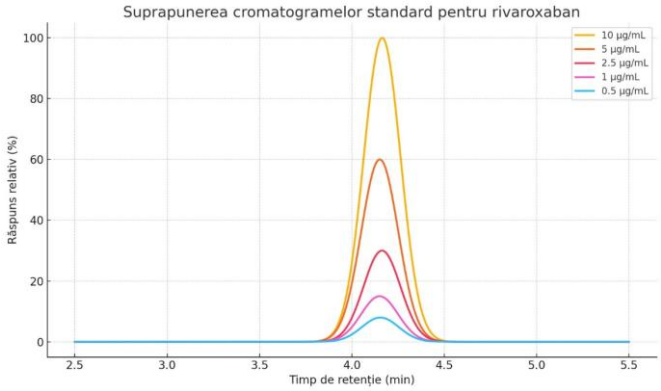

Figure 3. Representative illustration related to SCF processing, HPLC analytics, or dissolution workflow.

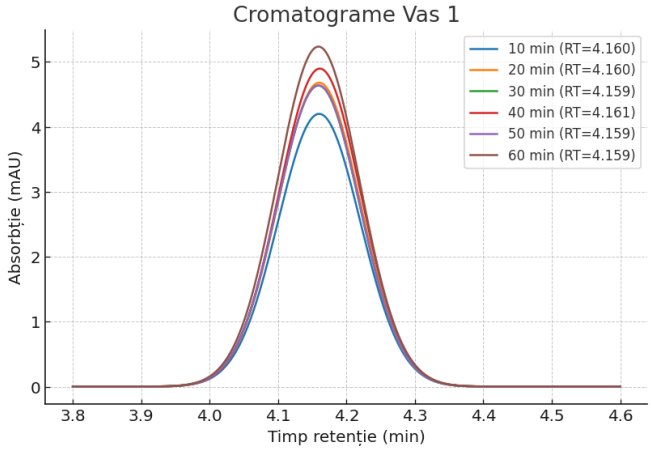

Figure 4. Representative illustration related to SCF processing, HPLC analytics, or dissolution workflow.

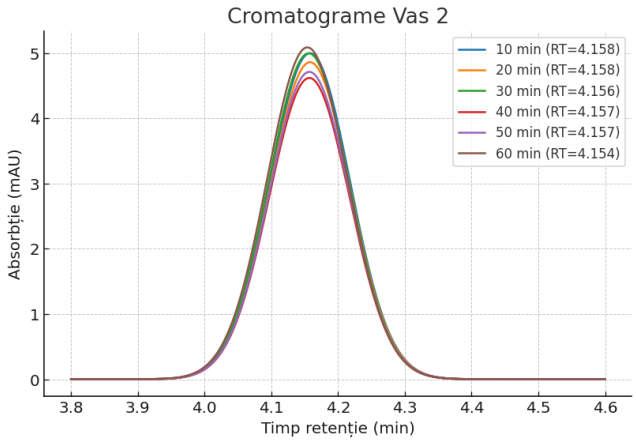

Figure 5. Representative illustration related to SCF processing, HPLC analytics, or dissolution workflow.

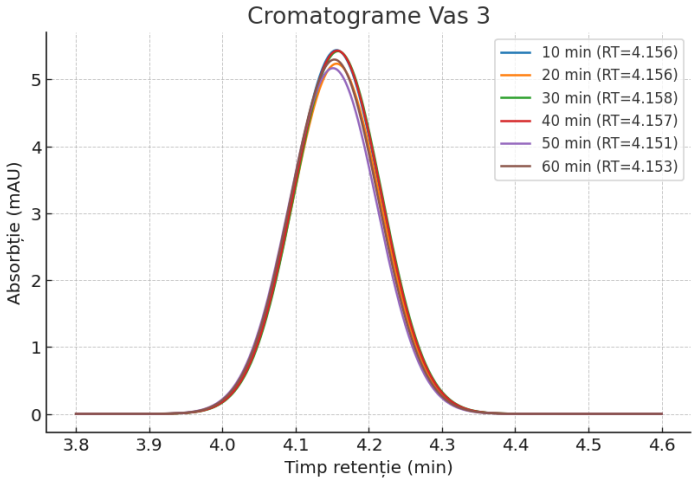

Figure 6. Representative illustration related to SCF processing, HPLC analytics, or dissolution workflow.

Supercritical fluids laboratory setup

The dissertation documents a laboratory-scale scCO₂ platform comprising a CO₂ cylinder, a high-pressure two-stage compressor (up to ~300 bar), high-pressure tubing and fittings, a pressure-rated viewing cell (~100

mL) with stainless-steel head and seals, pressure sensors, an injection/nozzle system, and an expansion/collection chamber. This setup underpins future nanocrystallization and encapsulation experiments.

Future work

Planned work includes (i) preparation of SCF-based rivaroxaban prototypes (nanocrystals, amorphous solid dispersions), (ii) dissolution testing in biorelevant media (e.g., fasted/fed states), (iii) stability studies under ICH-like conditions, and (iv) exploratory in vivo/IVIVC efforts to connect in vitro dissolution to pharmacokinetics.

Key quality attributes include assay accuracy, content uniformity, clarity (absence of visible particulates), and a vehicle-appropriate pH. Prospective stability should consider accelerated/long-term storage with periodic visual and assay checks. Method documentation and traceability support future validation and technology-transfer steps.

4. CONCLUSIONS

Within the scope of the dissertation, the HPLC-supported dissolution method proved robust, sensitive and discriminatory for rivaroxaban in a restrictive medium. The theoretical and infrastructural foundation for SCF-based processing provides a credible path to increase dissolution rate and, ultimately, oral exposure for poorly soluble, high-permeability drugs. The method described here establishes the analytical baseline for comparing forthcoming SCF-derived prototypes.

BIBLIOGRAPHY:

1. World Health Organization / FDA / EMA guidance on bioavailability and bioequivalence (as cited in the dissertation).
2. Ghid privind investigarea biodisponibilității și bioechivalenței, Ordin 1177/2004 (România).
3. Basit A.W., Gaisford S. (2020). Pharmaceutical Formulation: The Science and Technology of Dosage Forms. Wiley.
4. Shargel L., Wu-Pong S., Yu A.B. (2015). Applied Biopharmaceutics & Pharmacokinetics. McGraw-Hill.
5. Rowland M., Tozer T.N. (2018). Clinical Pharmacokinetics and Pharmacodynamics. Wolters Kluwer.
6. McHugh M.A., Krukonis V.J. (2013). Supercritical Fluid Extraction: Principles and Practice. Butterworth-Heinemann.
7. Reverchon E., De Marco I. (2019). Supercritical Fluid Processing of Pharmaceuticals. The Journal of Supercritical Fluids, 143, 75–89.
8. Knez Ž., Leitgeb M. (2015/2021). Industrial applications and encapsulation using $scCO_2$. Chemical Engineering Journal; Journal of Supercritical Fluids Research.
9. Kiran E., Debenedetti P.G. (2019). Supercritical Fluids: Fundamentals and Applications in the Pharmaceutical Industry. Annu Rev Chem Biomol Eng, 10, 321–347.
10. Wang T., Zhang Y., Yuan Y. (2020–2022). Advances in SCFs for drug formulation/dissolution control. Int J Pharm; Adv Drug Deliv Rev; IJPharm 613, 120-132.
11. Skerget M., Knez Ž., Habulin M. (2018–2019). Encapsulation of biopharmaceuticals using SCFs. J Pharm Sci; Biotechnology Advances.
12. Necula S.E. (2025). Optimizarea biodisponibilității. Dezvoltarea metodei analitice de testare a dizolvării Rivaroxabanului (Disertație), UTM – Facultatea de Farmacie.

CURRENT PERSPECTIVES IN THE MICROBIOLOGICAL DIAGNOSIS OF DERMATOPHYTOSES

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DOI: https://doi.org/10.66793/titum19proceeding16

Abstract

Globally, dermatophytosis affects approximately 25% of the population, being a category of cutaneous mycoses that affect the skin and nails with a varied epidemiology, influenced both by geographical location and by some populations themselves. The main etiological agent in the case of dermatophytosis is the species Trichophyton rubrum. Although in general the lesions in dermatophytosis are superficial, limited to the keratinized layer, cases of mycoses in which the etiological agent is a dermatophyte fungus are increasingly being reported in which layers such as the dermis or hypodermis are invaded, especially in people with a immunosuppressed immune system.

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Keywords*: cutaneous fungal infection; dermatophyte, tinea; fungi, mycological diagnosis.*

1. INTRODUCTION

Dermatophytosis (dermatophyte infection, tinea, ringworm) is a superficial cutaneous mycosis affecting the skin, nails and hair, caused by dermatophyte fungi [2,4,5,8]. Following recent multilocus phylogenetic studies, the taxonomy of dermatophytes has been revised such that species that were previously classified in the three known genera (*Epidermophyton*, *Microsporum* and *Trichophyton*) are now distributed in several genera, respectively: *Arthroderma*, *Epidermophyton*, *Lophophyton*, *Microsporum*, *Nannizzia*, *Paraphyton* and *Trichophyton* [7].

Depending on the location on the body surface, dermatophytosis can be found under different names, namely: tinea pedis (on the foot), tinea cruris (inguinal intertrigo), tinea corporis (affects glabrous skin), tinea barbae (on the chin and mustache), tinea faciei or sycosis (on the face), tinea capitis (can affect the scalp, eyebrows, eyelashes), tinea manuum (on the hand), tinea unghium (on the nails), tinea favosa (favus) and tinea imbricata or tokelau [5,12,13,17].

For a quality laboratory diagnosis, the way in which the sampling is carried out and the amount of material taken are very important. The material taken should be sufficient for both direct microscopy (important in the case of immunosuppressed patients for a rapid diagnosis) and for performing culture in the laboratory. Knowledge of additional data about the patient such as the appearance and location of lesions on the body surface, whether he has come into contact with an animal or whether he has traveled to certain areas, are useful in establishing the diagnosis of dermatophytosis [18].

In order to ensure proper sample collection, it is recommended that samples be collected before antifungal treatment begins, as well as removing any creams or lotions from the sample area using alcohol. Clean and sterile instruments, such as curettes, blunt scalpels, scissors, and forceps, should be used. A sterile swab can also be used to scrape the area from which the specimen was previously collected to ensure that all scales have been collected [18].

From the skin, in patients suspected of tinea corporis, the specimen is collected by scraping the lesion from the center to the edge.

From the foot, in the case of tinea pedis with blisters, the upper part of the blister is removed and collected; in the case of onychomycosis or tinea unguium, the subungual material or nail fragments are collected. From the scalp, in the case of tinea capitis or tinea favosa, the hairs are plucked or epilated if plucking them is not possible [18].

2. MATERIALS AND METHODS

In order to highlight the importance of the laboratory diagnostic in dermatophytosis, a meta-analysis of the specialized literature was conducted, mainly addressing the epidemiology, clinical manifestations and diagnosis of dermatophytosis, in the context in which the clinical manifestations may be similar to those of other dermatoses such as psoriasis or candidiasis, and laboratory examination is essential for identifying the etiological agent and choosing the appropriate treatment.

Laboratory diagnosis of dermatophytosis includes the following stages:

2.1.1. Direct microscopic mycological examination.
2.1.2. Inoculation of the collected materials on culture media (culture).

2.1. **Direct microscopic mycological examination**.
Direct microscopic mycological examination is performed using preparations mounted in a drop of KOH (potassium hydroxide) with a concentration of 10-20%, depending on the type of sample. To prevent crystallization and drying of the preparation, it is recommended to add glycerol.
For this examination, the sampled material (scales, subungual deposit, nail fragments, hairs) is placed on a glass slide, over which a drop of KOH is added and covered with a coverslip, the coverslip is gently pressed, avoiding the formation of air bubbles. The obtained preparation can be slightly heated and examined under a microscope after a contact time of 10-15 minutes, necessary for the degradation of keratin and clarification of the preparation (the time may vary depending on the reagent used, the amount of material taken and the degree of contamination) [10].

Interpretation of results:
In the case of a positive result, when scales or nail fragments are examined under a microscope, the following can be observed: hyaline, septate, branched hyphae and arthroconidia . Arthroconidia result from the fragmentation of hyphae, and their presence is definitive for making the diagnosis of dermatophytosis.

2.2. Inoculation of the collected materials on culture media (culture).
Regardless of the result of the direct microscopic mycological diagnosis, it is recommended to perform a culture for diagnosis and the susceptibility testing. To perform the culture, the samples (scales, subungual deposit, nail fragments, hairs) are inoculated on usual isolation media or on special culture media for dermatophytes, incubated at temperatures of 25-30ºC and observed for 4 weeks.
The identification of isolated strains is carried out by analyzing macroscopic and microscopic characters. When these are not sufficient for species identification, a series of tests can be used such as: the in vitro hair perforation test, the urea hydrolysis test, the rice growth test, tests for determining nutritional requirements and the thermotolerance test.

Microscopic characters

In the case of dermatophyte fungi, microscopic examination can show macroconidia, microconidia, arthroconidia, chlamydospores, "rocket" hyphae, spiral hyphae, "deer antler" hyphae and nodular organs (structures that may be present or absent depending on the genus and species of dermatophyte fungus). For example, the species *Epidermophyton flocossum* does not show microconidia. Very important for identifying the species, microscopic characters can be observed using extemporaneous preparations mounted in lactophenol blue (a) or by performing a culture on a slide (b).

(a). The extemporaneous preparation is the most used and is made by detaching a portion of the dermatophyte colony. The preparation is examined under a microscope with a 10x, 20x, 40x objective.
Another way in which the microscopic examination can be performed is the scotch tape mount technique, usually used for filamentous, sporulating fungi, it prevents the destruction of the spores, which happens to a large extent in the case of the tearing of the mycelial fragments. This technique is performed as follows: cut a small fragment of a transparent adhesive tape (scotch); lightly touch the surface of the colony with the sticky side of the tape, taking care not to touch the colony (for this, you can use tweezers or a wooden stick, which can be moistened with a small amount of alcohol to facilitate detachment from the tape fragment); place the

tape fragment on a slide in a drop of liquid (lactophenol blue is preferred); apply a drop of liquid over the adhesive tape, cover with a coverslip and examine under a microscope [18].
(b). The slide culture is used for the in situ observation of conidia (macroconidia ormicroconidia) and is performed by performing the following steps:
• placing a fragment of sterile glass in the shape of the letter V or U in the wet chamber, over which a sterile microscope slide is placed.
• placing a fragment of a culture medium (preferably a medium that stimulates sporulation) measuring approximately 1cm over the glass slide.
• the medium fragment is inoculated with the strain to be identified and may or may not be covered with a flamed coverslip.
• incubating the Petri dish at a temperature of 30˚C, until mycelial growth and sporulation are observed.
• extemporaneous preparations are made both by applying the slide, removed from the seeded medium fragment, to a drop of lactophenol blue and by directly observing the respective fragment under a microscope.
Conventional diagnostic methods are not always accurate, having some limitations, making it useful to use molecular diagnostic techniques in particular cases [18]. Table 1 present a summary of the advantages and disadvantages of the main diagnostic methods.

Table 1. Diagnostic methods and characteristics
Comparison of diagnostic methods for dermatophytosis

Methods	***Response time***	***Sensitivity***	***Main limitations***	***References***
Microscopy/culture	***Days-weeks***	***Variable***	***Slow, depends on expertise***	***(Aboul-Ella et al., 2020; Begum et al., 2020; Moskaluk & Vandewoude, 2022; Robert & Pihet, 2008; Pihet & Govic, 2017)***
PCR (various types)	***Hours-days***	***High Cost***	***requires equipment***	***(Petrucelli et al., 2020; Aho-Laukkanen et al., 2024; Gnat et al., 2020; Spanamberg et al., 2023)***
MALDI-TOF MS	***Minutes-hours***	***High***	***Limited database***	***(Chen et al., 2021; Machová et al., 2025)***
Rapid immunoassays	***Minutes***	***Good***	***Variable sensitivity***	***(Aboul-Ella et al., 2023)***
VOCs (fingerprint)	***Hours-days***	***Promising***	***Experimental stage***	***(Machová et al., 2025)***

Additional tests for the identification of dermatophyte species

1. In vitro hairperforation test.

The test is useful in the identification of dermatophyte fungal species, especially in differentiating *Tricophyton mentagrophytes* from *Tricophyton rubrum* species. It is performed by placing sterile hairs in a Petri dish, to which sterile distilled water (approximately 10ml), yeast extract (0.1ml) and a fragment of the dermatophyte colony are added. The Petri dish is incubated at 25ºC and examined weekly for approximately four weeks, by making extemporaneous preparations, preferably mounted in a drop of lactophenol blue.

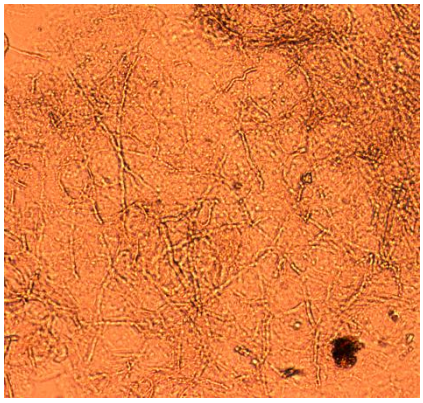
The test can also be performed by applying sterile hairs directly to the surface of the colony, incubating at 25ºC and examining them weekly for approximately four weeks, by making extemporaneous preparations, mounted in a drop of lactophenol blue.
Interpretation of results: in the case of a positive result, conical or wedge-shaped perforations are observed at the level of the hairs [18, 19].

Urea hydrolysis test
This test is used to differentiate Tricophyton mentagrophytes isolates from *Tricophyton rubrum*. The test can be variable in the case of certain species, Tricophyton mentagrophytes isolates are urease-positive while *Tricophyton rubrum* isolates, depending on the type to which they belong, can be negative or positive.
The fluffy type of *Trichophyton rubrum* is urease-negative while the granular type of *Trichophyton rubrum* isolates are urease-positive [18]. The urea hydrolysis test is also variable for other strains.

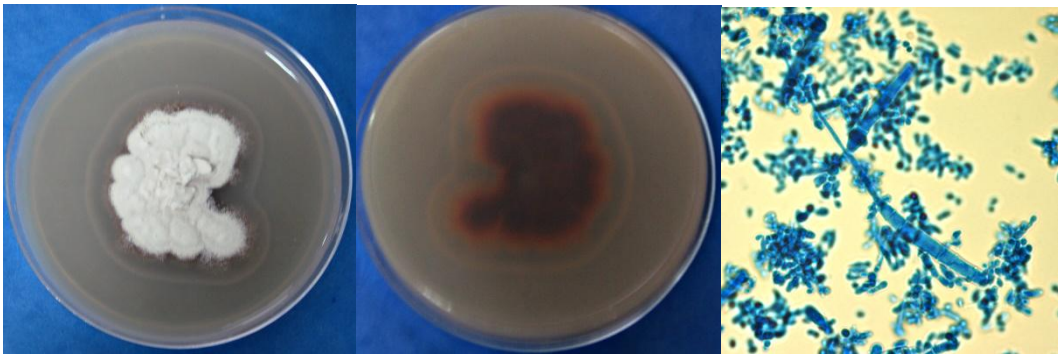
The test is performed by inoculating a colony fragment onto urea medium and incubating at 25º-30ºC for 7-8 days. It is tested in parallel with an uninoculated medium control and examined at 2-3 day intervals to observe whether the color of the culture medium has changed.
Interpretation of results:
In the case of a positive result, the color of the medium changes from light yellow to red-violet [18, 19].

3. RESULTS AND DISCUSSION

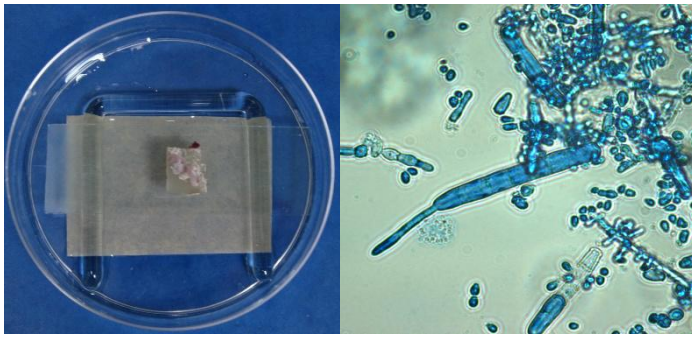
Diagnosis of dermatophytosis has evolved significantly in recent years, moving from laborious and slow methods to rapid and accurate molecular techniques. This change is essential for the effective treatment and prevention of the spread of fungal infections in humans and animals.
In the following images, you can see some of the macroscopic and microscopic characteristics of one of the most commonly isolated species of dermatophyte fungi with clinical importance, namely: *Trichophyton rubrum*.



Direct preparation mounted in 10% KOH, dermatophytosis scales of the trunk, 400X



***Trichophyton rubrum*, PDA medium; A-colony avers; B-colony reverse;**
C- macroconidia and microconidia, lactophenol cotton blue, 400x



***Trichophyton rubrum*; A- slide culture;**
B-macroconidia and microconidia, lactophenol cotton blue, 400x

5. CONCLUSIONS

For a positive result, the diagnosis-treatment correlation is important, through which the identification of the dermatophyte fungal species agent guides the choice of antifungal used in therapy, knowing that infections caused by *T. rubrum* species respond well to treatment with terbinafine and itraconazole. Also, to monitor the effectiveness of the chosen treatment, it is recommended to repeat the microscopic examination and perform the culture.
Traditional methods include direct microscopic examination and culture on specific media, which remain the gold standard but require up to 4 weeks to identify the etiological agent. These methods are inexpensive and accessible, but have variable sensitivity and specificity, depend on the experience of the personnel and do not always allow identification to the species level [1,4,14].

Molecular techniques, especially PCR (conventional, real-time, multiplex), have revolutionized diagnosis, providing results within hours and a sensitivity far superior to culture. Commercial tests (e.g. DermaGenius) and in-house methods cover most relevant species, reducing turnaround times from weeks to 16 hours [2,9, 17]. Other modern methods include microarrays (e.g. DendrisCHIP®), MALDI-TOF MS (mass spectrometry), and rapid immunochromatographic assays, each with specific advantages and limitations [1,6,15].

Method selection factors and future prospects. The choice of method depends on resources, urgency, the target species spectrum and the experience of the laboratory. Molecular methods are becoming increasingly accessible and can supplement or even replace classical diagnosis, but require extensive databases and standardization [4,14,17,20]. Innovative approaches are also being developed, such as identification based on volatile organic compound (VOC) fingerprinting [15].

REFERENCES

1.Aboul-Ella H, Hamed R, Abo-Elyazeed H. Recent trends in rapid diagnostic techniques for dermatophytosis. *International Journal of Veterinary Science and Medicine*. 2020;8:115–123. doi:10.1080/23144599.2020.1850204.
2.Aho-Laukkanen E, Mäki-Koivisto V, Torvikoski J, Sinikumpu S, Huilaja L, Junttila I. PCR enables rapid detection of dermatophytes in practice. *Microbiology Spectrum*. 2024;12:. doi:10.1128/spectrum.01049-24.
3..Al Otaibi M F, AlSharhan F, AlRujaib F, et al. Dermatophytosis in a Healthy Adolescent: A Report of Terbinafine-Resistant Trichophyton indotineae Infection in Kuwait. Cureus 17(5): e84108. doi:10.7759/cureus.84108.2025.
4.Begum J, Mir N, Lingaraju M, Buyamayum B, Dev K. Recent advances in the diagnosis of dermatophytosis. *Journal of Basic Microbiology*. 2020;60:293–303. doi:10.1002/jobm.201900675
5. Chanyachailert, P., Leeyaphan, C., Bunyaratavej, S. Cutaneous Fungal Infections Caused by Dermatophytes and Non-Dermatophytes: An Updated Comprehensive Review of Epidemiology, Clinical Presentations, and Diagnostic Testing. J Fungi (Basel). 2023.

6. Chen, J., Zheng, F., Sun, X., Gao, H., Lin, S., & Zeng, Y. (2021). The qualitative accuracy of clinical dermatophytes via matrix-assisted laser desorption ionization-time of flight mass spectrometry: a meta-analysis.. Medical mycology. https://doi.org/10.1093/mmy/myab049
7. de Hoog, G.S., Dukik, K., Monod, M., Packeu, A. Stubbe, D. M. Hendrickx, *et al*. Toward a novel multilocus phylogenetic taxonomy for the dermatophytes. Mycopathologia, 2017.

8. Deng R, Wang X, Li R: Dermatophyte infection: from fungal pathogenicity to host immune responses. Front Immunol. 14:1285887. 10.3389/fimmu.2023.1285887. 2023.
9. Gnat, S., Łagowski, D., Nowakiewicz, A., Dyląg, M., Osińska, M., & Sawicki, M. (2020). Detection and identification of dermatophytes based on currently available methods – a comparative study. Journal of Applied Microbiology, 130. https://doi.org/10.1111/jam.14778
10. Harel F, Robert-Gangneux F, Gangneux J, Guegan H. Monocentric evaluation of the Novaplex dermatophyte multiplex qPCR assay in the diagnosis of dermatophytoses. *Journal of Clinical Microbiology*. 2024;62:. doi:10.1128/jcm.00894-24.
11. Hill R, Caplan A, Elewski B, Gold J, Lockhart S, Smith D, et al. Expert panel review of skin and hair dermatophytoses in an era of antifungal resistance. *American Journal of Clinical Dermatology*. 2024;. doi:10.1007/s40257-024-00848-1.
12. Lanternier, F.; Pathan, S.; Vincent, Q.B.; Liu, L.; Cypowyj, S.; Prando, C.; Taibi, L.; Ammar-khodja, A.; Stambouli, O.B.; Guellil, B.; et al. Deep Dermatophytosis and Inherited CARD9 Deficiency. N. Engl. J. Med. 2013, 369, 1704–1714
13. Leung AK, Lam JM, Leong KF, Hon KL: Tinea corporis: an updated review. Drugs Context. 2020, 9:2020-5-6. 10.7573/dic.2020-5-6.

14. Machová L, Gaida M, Semerád J, Kolařík M, Švarcová M, Jašica A, et al. First step on the way to identify dermatophytes using odour fingerprints. *Mycopathologia*. 2025;190:. doi:10.1007/s11046-024-00905-7.
15. Moskaluk A, Vandewoude S. Current topics in dermatophyte classification and clinical diagnosis. *Pathogens*. 2022;11:. doi:10.3390/pathogens11090957.
16. Padhye, A. A., Summerbell, R. C., 2005, *The dermatophytes*; in Medical Mycology; Toplay & Wilson's; ten eddition cap.13: 220-240, 2005.
17..Petrucelli, M. F., Heinzen de Abreu, M., Michelotto Cantelli, B.A., Gonzalez Segura,G., Garcia Nishimura, F., T.A., Bitencourt, Marins,M., Fachin, A.L., Epidemiology and Diagnostic Perspectives of Dermatophytoses. J. Fungi. 2020.
18.http://www.mycology.adelaide.edu.au/Laboratory_Methods/Microscopy_Techniques_and_Stains/cellotape.html).
19. http://www.doctorfungus.org/Mycoses/human/other/skin_index.php#Dermatophytosis
20. Yapalak, Z., & Atılan, K. Laboratory Diagnosis of Medically Important Dermatophytes: Traditional Methods and New Developments. Journal of Molecular Virology and Immunology. 2023.

ANALYSIS OF SOME TYPES OF HONEY FROM DIFFERENT ROMANIAN AREAS

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DOI: https://doi.org/10.66793/titum19proceeding17

ABSTRACT

Honey is a natural product of high nutritional and biological value, whose composition and properties depend on the source flora, ecological conditions and geographical regions of origin. In the context of increasing interest in the authenticity and quality of bee products, the scientific characterization of local honey becomes essential for certification, traceability and prevention of counterfeiting. This study presents a scientific characterization of honey from various regions of Romania, focusing on authenticity, quality and floral specificity. During the study, physicochemical, organoleptic and bioactive properties were analyzed, as well as pollen content to identify the predominant flora and confirm the regions of origin. The results highlighted honey's compliance with national standards, providing a basis for certification, traceability and prevention of counterfeiting. The study highlights the biological value and healthy potential of native honey, supporting its promotion as a product of controlled origin.

Keywords: *Honey, health, sugar.*

INTRODUCTION

For over six millennia, honey has been recognized as both food and medicine, having a rich written history. The close connection between honey and human life is evidenced by the numerous archaeological remains from the Mesolithic period, as well as the historical documents of the great ancient civilizations, including the Egyptian, Sumerian, Chinese, Greek, and Roman ones. Bee honey is a complex natural product, resulting from floral nectar, which is enriched by bees (Apis mellifera L) with specific substances, from the secretions of their glands. [1]

Honey has a sweetening capacity superior to sugar, but with fewer calories. Honey contains glucose, fructose and, in small quantities, maltose, sucrose, etc. [2][3] The nutritional value of honey is extremely important due to its high content of directly assimilable sugars (glucose, fructose), which provides the body with a quick source of energy. The sugars in honey have a hepatoprotective effect: glucose is quickly available in the blood for energy, and fructose is stored in the liver (energy reserve that can be quickly mobilized). [4]

Honey contains a variety of minerals, vitamins, antioxidants, amino acids, and enzymes. Vitamins B6, thiamine, riboflavin and pantothenic acid, vitamin C, minerals such as iron, calcium, copper, magnesium, zinc, etc. A unique antioxidant found in honey is pinocembrin, with antiseptic and antibiotic properties, which give it a strong antibacterial action. [2][3] The polyphenols present in honey act as powerful antioxidants, helping to prevent and treat various ailments and strengthen the immune system. [4]

Honey is frequently recommended for increasing resistance to mental and intellectual fatigue, having a beneficial impact in cases of asthenia, convalescence, anorexia, growth retardation in children. [5][6] Numerous studies demonstrate that honey provides nutritional benefits, such as preventing fatigue and improving athletes' performance. It is useful in treatments for chronic bronchitis, allergies, rheumatic diseases, digestive diseases, insomnia, etc.

The chemical composition of honey is influenced by factors such as the nature of the raw material (nectar or manna), its quality and abundance, climatic conditions, bee exploitation methods, harvesting techniques, and conditioning and preservation processes. The composition includes groups of inorganic and organic substances, solubilized or dispersed in the water present in honey. The water content of honey influences the preservation and crystallization process, varying under optimal conditions between 17% and 18%. Honey with a high glucose content is more hygroscopic, which can shorten the shelf life. [7]

Among the organic acids contained in honey are predominantly gluconic acid, with a preservative and bactericidal role, as well as acetic, lactic, malic, succinc, butyric, citric, formic acid, etc. The proteins in honey are found in small amounts (0.15–0.8%), and honey contains enzymes such as amylase, oxidase, catalase, maltase, phosphatase, glucosidase, etc. [7] The most important carbohydrates are the monosaccharides fructose and glucose, as well as the disaccharide sucrose, and the type of flower influences the ratio between them. [6] Aromatic compounds, including polyphenols (flavonoids such as quercetin, luteolin, kaempferol, apigenin, galangin), phenolic acids, and derivatives, impart the taste of honey. [8]

The color of honey varies significantly depending on the type of honey, from colorless to dark shades, predominantly yellow, with rare brown or green tones. The flavor is influenced by the plant species from which the nectar comes and the content of essential oils. The taste is predominantly sweet, impacted by compounds such as gluconic acid, proline, and tannins; fermentation can generate a sour taste. Viscosity depends on water content and environmental temperature, and hygroscopicity allows moisture to be absorbed from the air, influencing the texture and consistency of honey. [4][9]

Honey has beneficial effects on the digestive system, helping to fight constipation and treat certain stomach and intestinal conditions. [4] Bee products are an essential source of energy, also integrated into cosmetic treatments due to their antioxidant, toning and soothing properties. [8]

Honey has the ability to inhibit the growth of microorganisms and fungi, being especially effective against gram-positive bacteria. Its antibacterial activity depends on the content of water and glucoso-oxidase, which generates hydrogen peroxide. Honey also contains antioxidant substances such as catalase, ascorbic acid, flavonoids, phenolic acids, and carotenoid derivatives. [10][11]

Experimental and clinical studies have demonstrated various health effects [12]: increases in the antioxidant activity of plasma, vitamin C (+47%), β-carotene (+3%) and glutathione reductase (+7%); immunosuppressive action, reducing sensitivity to allergens; increases in monocytes (+50%), iron (+20%), copper (+33%) and slight increases in lymphocytes, eosinophils, zinc, hemoglobin and platelets; decreases in ferritin (-11%), IgE (-34%), AST (-22%), ALT (-18%) and creatinine kinase (-33%); inhibition of Helicobacter pylori and protection against gastric ulcers; favoring the development of bifidobacteria and Lactobacillus in the intestine; honey consumption compared to glucose and fructose-based sweeteners reduced insulin and C-reactive protein levels, lowering total cholesterol, LDL-C and triglycerides, slightly increasing HDL-C; honey contains metabolites of nitric oxide (NO) with a cardiovascular protective role. [12][13]

The therapeutic effects of different types of honey include [5]:

- Linden honey: reduces fever and gastric pain, prevents migraines, useful in pneumonia, bronchial asthma and nervous states
- Acacia honey: soothing and tonic
- Honey: antihemorrhagic and toning
- Sunflower honey: useful in bronchitis and stomach ailments.

The consumption of honey has shown beneficial effects in hepatitis A, significantly reducing the activity of alanine-amino-transferase and increasing the production of bilirubin. It also supports patients undergoing radiotherapy in reducing mucus and maintaining body weight. [12]

However, there are minor risks: the presence of Clostridium botulinum spores can be dangerous in children under one year of age, with rare cases of infant botulism being reported.

MATERIALS AND METHOD

The quality control of honey was carried out on several batches, through organoleptic examinations and physico-chemical analyses, including the determination of the content of water, sucrose, invert sugar and hydroxy-methyl-furfurol (HMF), as well as the identification of the assortment through pollen analysis.

Analyzed samples

Eight types of honey sold from various regions of the country were investigated: polyfloral honey from the Craiova area, rapeseed honey from Vâlcea, acacia honey from a beekeeping store, polyfloral honey from the store, sunflower honey from Prahova, linden honey from the store, as well as rapeseed and polyflora honey from the Negrești Călărași area.

Microscopic pollen analysis

The examination of the pollen granules was carried out with the Motic Panther microscope. Each type of pollen has distinct characteristics (shape, size), which allow the identification of the floral source and the classification of honey as monoflora or polyflora. This method is essential for authentication and evaluation of honey quality.

Organoleptic determinations and pH

The color of the honey was visually evaluated on 10–15 g of sample, and the smell and taste by smell and tasting, to identify the source plant. The smell has been described as "pleasant", "sweet", "weakly flavoured" or "sour", "bitter", "astringent". [13][14]

The determination of the pH, carried out by using pH paper for a quick estimate, allowed the evaluation of the physicochemical and biological properties.

Determination of Invert Sugar (Fihe Reaction)

5 g of honey were weighed, crushed in a mortar and transferred to a porcelain capsule with 2–3 ml of ethyl ether in the niche. After the ether evaporated, a few drops of hydrochloric resorcin were added to highlight the invert sugar.

Identification of add-ons

- **Starch, flour and derivatives:** 5 g of honey were dissolved in 5 ml of distilled water, brought to a boil and cooled, then drops of iodine solution in 1% potassium iodide were added.
- **Gelatin or glue:** Honey diluted 1:2 has been mixed with a few drops of 5% tannin solution. The appearance of a floclonous precipitate indicates the presence of the gelatinous substance.

RESULTS AND DISCUSSIONS

Microscopic pollen analysis

The identification of pollen granules was carried out with the help of the Motic Panthera microscope, for samples of honey from different regions.

In the polyfloral honey samples (Fig. 1–4), the pollen granules showed various shapes, from circular to slightly elongated, reflecting the floral variety. Rapeseed honey contains predominantly *Brassica napus* pollen, with almost spherical granules, smooth surface and small size. Some blades have been stained in the microbiology lab to more clearly differentiate the types of pollen.

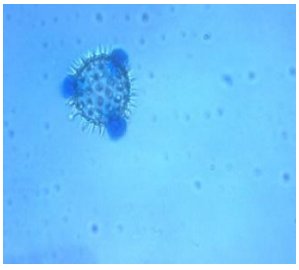


Fig.1 Polyfloral honey pollen granules Prahova

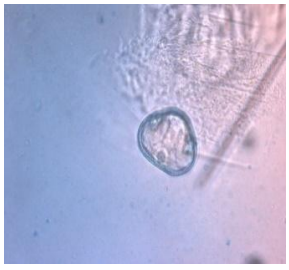
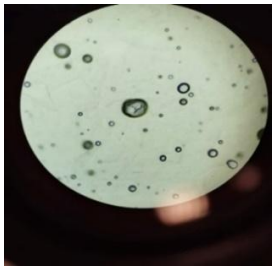


Fig.2 Rapeseed pollen granules Vâlcea



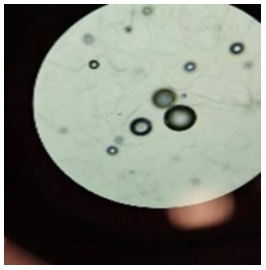


Fig.3/4 Polyflora honey pollen granules Prahova

The granules of linden honey (Fig. 5) have a slightly elongated shape, with three small characteristic shadows. The pollen in sunflower honey (Fig. 6) is round, with "fangs" on the edges, and that in polyfloral honey (Fig. 7–8) shows obvious morphological diversity.

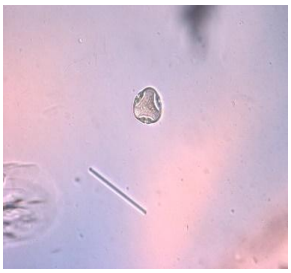


Fig.5 Linden honey pollen granules

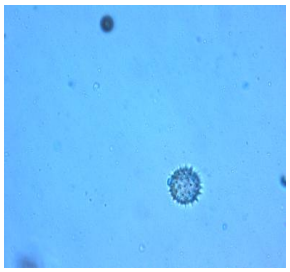
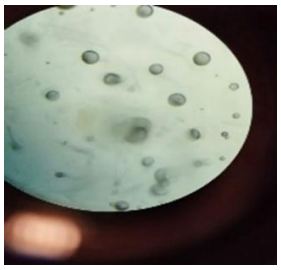


Fig.6 Sunflower honey pollen granules



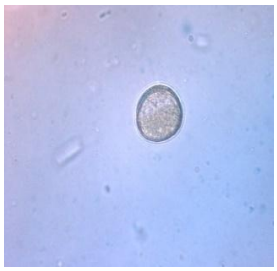


Fig.7/8 Polyfloral honey pollen granules

In the samples from Prahova and Călărași (Fig. 9–12), the pollen grains of sunflower (*Helianthus annuus*) are larger, oval-triangular, with a spiny surface. Linden granules come from commercial samples and have distinctive characteristics that allow the precise identification of the botanical origin of honey.

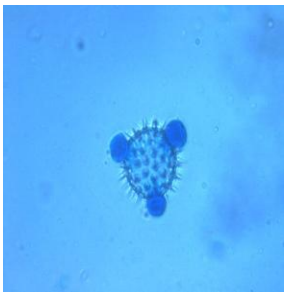
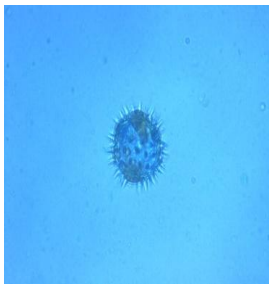



Fig.9/10 Pollen granules, honey, sunflower, linden trees Prahova/ Călărași

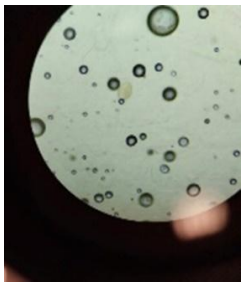
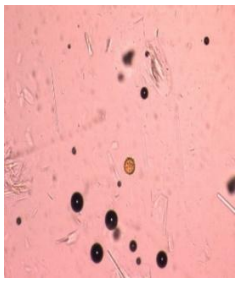



Fig. 11/12 Pollen granules, linden flowers shop

Pollen granules from linden honey (*Tilia spp.*) are spherical or slightly elliptical in shape, with a finely reticulated surface and medium dimensions (Fig. 13–14). The polyfloral honey from Călărași (Fig. 15–16) reflects a great floral diversity, with granules varied in shape and size, with local species such as clover, alfalfa and field weeds predominating.

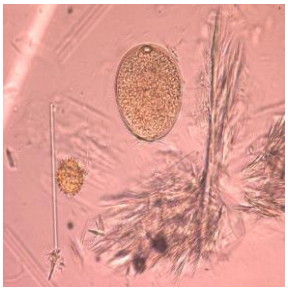
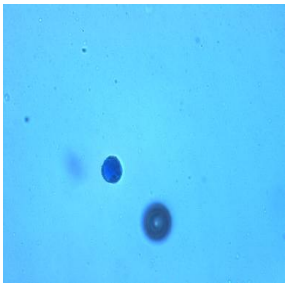



Fig.13/14 Pollen granules honey lime blossom

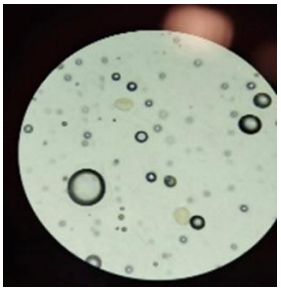
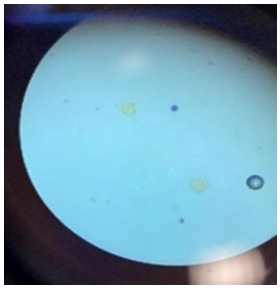



Fig.15/16 Pollen granules honey polyflora Călărași

Organoleptic determinations and pH

The organoleptic examination highlighted the typical characteristics of each type of honey:

- **Rapeseed honey:** very light color, fine and creamy texture, sweet taste, slightly bitter, weak aroma.
- **Linden honey:** golden-yellow color, intense floral aroma, sweet, slightly menthol taste.
- **Polyfloral honey:** light yellow color, complex aroma, balanced, sweet taste, with distinct floral notes.
- **Sunflower honey:** intense yellow, almost orange color, specific aroma, slightly fruity, sweet taste, with a sour tinge.

The pH determination showed values between 3.4 and 4.5:

- Rapeale: 4.0–4.5
- Lime: 3.5–4.2
- Polyflora: 3.5–4.2
- Sunflower: 3.4–3.8

The values confirm the slightly acidic nature of the honey, which is essential for stability and preservation, and comply with quality standards.

Determination of Invert Sugar and HMF

The analysis of invert sugar (Fiehe Reaction) did not reveal its presence in the analyzed samples, indicating that the honey was not subjected to adulteration by the addition of invert sugar.

Hydroxymethylfurfural (HMF), a compound formed by the degradation of hexoses under thermal and acidic conditions, was not detected in the samples analyzed, confirming that the honey did not undergo processes of excessive heating or adulteration by the addition of sugary syrups.

Identification of add-ons

As for the addition of starch/flour, no blue coloration was observed, indicating the absence of addition. With regard to the addition of gelatin or glue, no flake precipitation was detected, confirming the absence of the addition of gelatinous substances.

The analysis of the pollen content showed that linden honey has the richest pollen content, which is to be avoided for people with pollen allergy. The pH composition, organoleptic characteristics and lack of artificial additives confirm the authenticity and high quality of the analyzed products.

The honey studied complies with quality and food safety standards, and the methods used provide a solid basis for certification, traceability and prevention of counterfeiting. The standardization of testing and certification processes contributes to the protection of consumers and the correct use of local honey.

The work was carried out on a limited number of samples from certain regions, which may restrict the generalization of the results. Pollen analysis, although useful for identifying botanical origin, may be incomplete for polyfloral honey, and monitoring of storage conditions has not been carried out, which could influence chemical parameters. The study also focused on authenticity and chemical quality, without directly assessing the health effects of consumers.

CONCLUSIONS

The analysis of honey from different regions of Romania highlighted specific characteristics of each floral type, such as pollen content, pH and organoleptic properties, confirming the authenticity and quality of the studied products. Linden honey was found to be rich in pollen, being less recommended for people with allergies, and all samples were found to be free of additives or falsifications.

The results underline the importance of standardizing the testing and certification process, which guarantees consumer safety and maintaining the quality of Romanian honey. Overall, local honey presents itself as a valuable product for health, the effective recovery of which requires continuous research, the protection of local beekeeping and the promotion of strict quality standards.

Bibliography

1 S. Lazăr, C. O. Vornicu, Bees and their health, Alpha Publishing House, 2009.
2 Stefan Lazăr, O.C. Vornicu, Apicultura, Alpha Publishing House, 2007.
3 Bratu I, Georgescu C, Aspects regarding the composition and quality of some varieties of honey in Romania in relation to the norms provided by Codex Alimentarius, Sibiu, vol. IV.
4 http:/eur-lex.europa.eu/ro/index.htm
5 Valentin Stroescu, The Pharmacological Bases of Medical Practice, Medical Publishing House, 1997
6 Bianchi EM: Honey: Its importance in children's nutrition. Amer Bree J, 1977
7 https://www.miereaalbine.ro/calitatea-mierii-si-clasificarea-ei/

8 American Honey Board: Honey Nutrition and Health. National Honey Board-2005, www.honeystix.com/HoneyStix/compendium.pdf, assed June 13, 2007.
9 http://labrom.ro/analize-de-laborator-pentru-miere/
10 http://wwwapimondiafoundation.org
11 Valentin Stroescu, Pharmacology, revised and added third edition, All Bucharest Publishing House, 1999
12 Al -Quassemi R, Robinson RK: Some special nutritional properties of honey – a briefreview. NutrFood Sci, 2003
13 http://miereaurie.ro/verificarea-mierii
14 Vasile Stănescu, Hygiene and Food Control, Practicum sanitary veterinary, Romania of Tomorrow Foundation Publishing House, Bucharest, 1998

EVALUATION OF ESSENTIAL OIL FORMULATIONS FOR INSECT REPELLENCY: CORRELATING CHEMICAL COMPOSITION WITH EFFICACY IN SUSTAINABLE INSECT CONTROL

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DOI: https://doi.org/10.66793/titum19proceeding18

Abstract:

In recent decades, the use of natural products for pest control has gained increasing attention as an eco-friendly alternative to synthetic pesticides, which pose environmental and health risks. Essential oils, complex mixtures of volatile organic compounds derived from aromatic plants, exhibit notable repellent and insecticidal properties. Their biological activity depends on chemical composition, purity, and extraction methods, which can be precisely analyzed using techniques.
This study presents a physico-chemical analysis of selected essential oils and correlates their chemical profiles with observed biological effects on insects. Three formulations were tested, exploring the correlation between chemical structure and biological effects on insects with significant variability in repellency based on formulation type and carrier medium. Oil-based compositions, particularly those containing citronella, peppermint, cedar, and tea tree oils, demonstrated superior persistence compared to aqueous ones.
The results confirm findings from existing literature regarding the efficacy of oils such as citronella, tea tree, peppermint, and cedar in repelling insects. Furthermore, the research highlights the importance of formulation type, environmental conditions, and potential synergistic interactions between components.
Future studies should extend testing to multiple insect species, assess long-term repellency, and explore optimized pharmaceutical formulations to develop safe, effective, and sustainable natural insect repellents.

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Keywords: *Essential oils; natural repellents; insect control; physico-chemical analysis; volatile compounds; eco-friendly pesticides; biological activity; sustainable formulations; neurotoxic mechanisms*

1. INTRODUCTION

Over the past decades, the need for natural alternatives to chemical pesticides has become increasingly urgent. Conventional insecticides, while effective, often pose ecological and toxicological risks—contaminating water, soil, and food chains, and potentially affecting non-target organisms, including humans. Essential oils, complex mixtures of volatile organic compounds derived from plants, offer a natural solution grounded in biocompatibility and sustainability.

Essential oils are primarily composed of terpenes, aldehydes, alcohols, esters, ketones, and phenols—each contributing distinct biological activities such as antimicrobial, antioxidant, and neurotoxic effects on pests. Their capacity to disrupt insect nervous systems, inhibit acetylcholinesterase, and interfere with olfactory receptors makes them valuable for pest deterrence and control. Furthermore, their biodegradability and low persistence in the environment position them as safer alternatives for both agricultural and domestic application

2. MATERIALS AND METHODS

1.1 Essential Oils

The study used a selection of commercially available essential oils of verified purity (>98%), extracted from plants known for their repellent and neuroactive properties. The following oils were included:

- Citronella (Cymbopogon nardus)
- Tea tree (Melaleuca alternifolia)
- Cedarwood (Cedrus atlantica)
- Peppermint (Mentha piperita)
- Clove (Syzygium aromaticum)
- Rosemary (Rosmarinus officinalis)
- Geranium (Pelargonium graveolens)

All oils were stored in amber glass bottles, tightly sealed, and kept at 4–8°C to minimize oxidation and preserve volatile components.

1.2. Vehicles and Additives

To evaluate the influence of formulation type on repellency, three different bases were prepared:

- Spray I: Apple vinegar (aqueous base)
- Spray II: Ethanol 70% (hydroalcoholic base)
- Spray III: Fractionated coconut oil (lipophilic base)

Each formulation contained an identical concentration of essential oils (10% v/v total oil content) mixed in equal parts. No synthetic stabilizers or emulsifiers were added to maintain a fully natural composition.

2. Preparation of Formulations

Each blend was prepared under aseptic conditions using magnetic stirring to ensure uniform dispersion. The essential oils were mixed with their respective carrier at room temperature (22 ± 2 °C) until homogeneity was achieved. Samples were then transferred to 50 mL dark glass spray bottles and labeled as Spray I, II, or III. Prior to testing, all samples were equilibrated for 24 hours to allow stabilization of the volatile fraction.

Physico-chemical profile of essential oils

The biological activity of essential oils (EOs) is intimately linked to their chemical structure. The major constituents—monoterpenes and sesquiterpenes—are responsible for the oils' volatility and bioactivity. For instance, compounds such as linalool, eugenol, citronellal, and thymol exhibit strong insecticidal or repellent effects. Their reactivity depends on molecular configuration, oxidation potential, and interaction with environmental variables like temperature, humidity, and light exposure. Physically, EOs are characterized by low density, high volatility, and solubility in organic solvents. Chemically, their stability is influenced by oxygen, light, and heat; improper storage leads to oxidation and degradation of active compounds. These physicochemical traits not only determine shelf life but also affect biological potency—hence the importance of rigorous analytical control.

Mechanisms of neurotoxic and repellent action

At the molecular level, many EO components act as neurotoxins for insects. They interfere with neurotransmission by inhibiting acetylcholinesterase (AChE), an enzyme responsible for hydrolyzing acetylcholine at synaptic junctions. The accumulation of acetylcholine results in overstimulation, paralysis, and eventual death of the insect. Phenolic monoterpenes such as thymol, carvacrol, and eugenol are particularly effective AChE inhibitors.

In addition, EOs modulate the octopaminergic and tyraminergic systems unique to invertebrates, influencing behavioral and sensory functions without affecting mammals. This selective toxicity is crucial for environmental safety. Studies reveal that exposure to compounds such as menthol, α-pinene, and β-caryophyllene disturbs the olfactory and ion channel function of insects, impairing their ability to locate hosts or mates.

From a behavioral perspective, repellency results from disruption of olfactory receptor neurons. Insects perceive volatile EO molecules as aversive stimuli, prompting avoidance behavior. Such mechanisms underline the dual role of essential oils—both as neurotoxic agents and behavioral deterrents.

Experimental evaluation of essential oil formulations

This study analyzed three formulations containing various essential oils and carrier bases to evaluate repellent efficacy against insect models under controlled laboratory conditions. The tested formulations included:

- Spray I – Aqueous base (apple vinegar) with mixed essential oils.
- Spray II – Ethanolic base with clove, rosemary, and geranium oils.
- Spray III – Oil base (fractionated coconut oil) containing citronella, tea tree, cedar, and peppermint oils.

Figure 1. Repellent effect for the 3 formulations – Circle test

3. RESULTS AND DISCUSSION

Results indicated that Spray III consistently produced the strongest and most prolonged repellency. The oil-based carrier enhanced adherence to surfaces and delayed evaporation, allowing a sustained release of active compounds. Spray II showed moderate protection, while Spray I—despite its rich oil content—had limited efficiency due to rapid volatilization in dry conditions.

The findings correspond closely with studies reported in Journal of Medical Entomology and Parasites & Vectors, where the vehicle medium significantly affected the longevity and bioavailability of volatile repellents. Oil-based carriers, such as coconut or mineral oils, not only improved adhesion but also extended the effective duration up to six hours, compared to less than two for aqueous bases.

The physico-chemical analysis confirmed that EO efficacy is highly dependent on both composition and formulation. Monoterpenes and phenolic compounds such as citronellal, linalool, and thymol were identified as key contributors to repellency and neurotoxic action. Their synergistic behavior, however, remains complex—certain combinations may enhance or diminish overall activity.

Environmental conditions—temperature, humidity, and air circulation—also play a decisive role. High temperatures accelerate volatilization, while humidity may enhance persistence by reducing evaporation rates. Consequently, future formulations should consider encapsulation or emulsification technologies to optimize stability and controlled release.

Beyond repellency, several tested oils demonstrated additional pharmacological properties: lavender and cedar exhibited calming effects; tea tree displayed antimicrobial and antifungal activity; peppermint provided analgesic and cooling sensations. These multifunctional attributes suggest potential for dual-purpose products—repellent and therapeutic.

4. CONCLUSIONS

This research reinforces the potential of essential oils as sustainable, safe, and effective agents in insect control. The correlation between chemical composition and biological efficacy emphasizes the need for analytical precision and formulation optimization.

Among the tested products, Spray III achieved the highest repellency due to its balanced oil blend and stable vehicle. These results align with global trends advocating for greener pest control technologies that minimize environmental impact while maintaining effectiveness.

Future studies should extend beyond laboratory conditions to real-field applications, incorporating multiple insect species and environmental parameters. Analytical monitoring (GC–MS, HPLC) of active component stability will be vital for product standardization. Moreover, exploring microencapsulation, polymer films, or emulsion systems may enhance protection duration and user safety.

Finally, interdisciplinary collaboration between chemistry, pharmacology, entomology, and dermatology will be crucial in transforming essential oils from promising natural resources into standardized, evidence-based bioinsecticides and repellent pharmaceuticals.

BIBLIOGRAPHY:

[1] M. Butnariu, "Bioactive Natural Volatile Oils," *Ann Clin Med Case Rep*, vol. 12, no. 2, pp. 1–6, 2023, [Online]. Available: https://acmcasereport.org/, accesat la data de 14.05.2025

[2] D. A. Murray and W. L. Lockhart, "Determination of trace volatile organic compounds in fish tissues by gas chromatography.," *J. Assoc. Off. Anal. Chem.*, vol. 71, no. 6, pp. 1086–1089, 1988.

[3] E. Wood and N. Bennett, "Changing theories, changing practice: Exploring early childhood teachers' professional learning," *Teach. Teach. Educ.*, vol. 16, pp. 635–647, Jul. 2000, doi: 10.1016/S0742-051X(00)00011-1.

[4] H. Sakauchi, H. Kiyota, S. Takigawa, T. Oritani, and S. Kuwahara, "Enzymatic resolution and odor description of both enantiomers of lavandulol, a fragrance of lavender oil.," *Chem. Biodivers.*, vol. 2, no. 9, pp. 1183–1186, Sep. 2005, doi: 10.1002/cbdv.200590088.

[5] A. U. Cruz-Castillo, L. M. Rodríguez-Valdez, J. Correa-Basurto, B. Nogueda-Torres, S. Andrade-Ochoa, and G. V. Nevárez-Moorillón, "Terpenic Constituents of Essential Oils with Larvicidal Activity against Aedes Aegypti: A QSAR and Docking Molecular Study," *Molecules*, vol. 28, no. 6, p. 2454, Mar. 2023, doi: 10.3390/molecules28062454.

[6] Z. Stojanović-Radić *et al.*, "Antistaphylococcal activity of Inula helenium L. root essential oil: eudesmane sesquiterpene lactones induce cell membrane damage," *Eur. J. Clin. Microbiol. Infect. Dis.*, vol. 31, no. 6, pp. 1015–1025, 2012, doi: 10.1007/s10096-011-1400-1.

[7] M. Machado *et al.*, "Activity of Thymus capitellatus volatile extract, 1,8-cineole and borneol against Leishmania species.," *Vet. Parasitol.*, vol. 200, no. 1–2, pp. 39–49, Feb. 2014, doi: 10.1016/j.vetpar.2013.11.016.

[8] G. N. Amzallag, O. Larkov, H. M. Ben, and N. Dudai, "Soil microvariations as a source of variability in the wild: the case of secondary metabolism in Origanum dayi post.," *J. Chem. Ecol.*, vol. 31, no. 6, pp. 1235–1254, Jun. 2005, doi: 10.1007/s10886-005-5283-4.

[9] N. J. Sadgrove, G. F. Padilla-González, and M. Phumthum, "Fundamental Chemistry of Essential Oils and Volatile Organic Compounds, Methods of Analysis and Authentication," *Plants*, vol. 11, no. 6, 2022, doi: 10.3390/plants11060789.

[10] W. Choochote *et al.*, "Repellent activity of selected essential oils against Aedes aegypti.," *Fitoterapia*, vol. 78, no. 5, pp. 359–364, Jul. 2007, doi: 10.1016/j.fitote.2007.02.006.

[11] L. S. Nerio, J. Olivero-Verbel, and E. Stashenko, "Repellent activity of essential oils: A review," *Bioresour. Technol.*, vol. 101, no. 1, pp. 372–378, 2010, doi: 10.1016/j.biortech.2009.07.048.

[12] S. J. Moore, N. Hill, C. Ruiz, and M. M. Cameron, "Field evaluation of traditionally used plant-based insect repellents and fumigants against the malaria vector Anopheles darlingi in Riberalta, Bolivian Amazon.," *J. Med. Entomol.*, vol. 44, no. 4, pp. 624–630, Jul. 2007, doi: 10.1603/0022-2585(2007)44[624:feotup]2.0.co;2.

[13] Y. Trongtokit, Y. Rongsriyam, N. Komalamisra, and C. Apiwathnasorn, "Comparative repellency of 38

essential oils against mosquito bites.," *Phytother. Res.*, vol. 19, no. 4, pp. 303–309, Apr. 2005, doi: 10.1002/ptr.1637.

[14] J. Govere, D. N. Durrheim, N. Du Toit, R. H. Hunt, and M. Coetzee, "Local plants as repellents against Anopheles arabiensis, in Mpumalanga Province, South Africa.," *Cent. Afr. J. Med.*, vol. 46, no. 8, pp. 213–216, Aug. 2000.

[15] A. Tawatsin, S. D. Wratten, R. R. Scott, U. Thavara, and Y. Techadamrongsin, "Repellency of volatile oils from plants against three mosquito vectors.," *J. Vector Ecol.*, vol. 26, no. 1, pp. 76–82, Jun. 2001.

QUALITATIVE ANALYSIS AND DETERMINATION OF TOXIC CONTAMINANTS IN DRINKING WATER FROM DIFFERENT SOURCES

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DOI: https://doi.org/10.66793/titum19proceeding19

Abstract:

The study evaluates the quality of drinking water from ten sources (six bottled brands, two tap water samples—filtered and unfiltered, and two wells) by determining key physico-chemical indicators and screening for toxic contaminants. The methods used included the JBL PRO Aquatest Combi Set Plus (NH_4) and rapid colorimetric procedures for pH, nitrate, nitrite, and ammonium, complemented by an electrolysis test for the qualitative assessment of dissolved ions. Results showed pH values within the acceptable range (6.5–8.0) for all samples, very low nitrate concentrations in bottled waters (<0.5–1 mg/L in reported cases), and a critical exceedance of nitrite in the deep-well sample (0.8 mg/L vs. ≤0.1 mg/L guideline), indicating sanitary non-conformity and unsuitability for consumption. Electrolysis revealed elevated ionic content and possible metal presence in some non-bottled samples.
The findings highlight the relative safety of bottled waters and the importance of routine monitoring, proper well isolation, maintenance of filtration systems, and compliance with WHO/EU and Romanian standards.

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Keywords: *drinking water, physico-chemical parameters, nitrates, nitrites, ammonium, heavy metals, WHO, EU, JBL Aquatest*

1. INTRODUCTION

Drinking water is a major determinant of public health. According to operational definitions in the field, safe water for human consumption must be colorless, odorless, free of pathogenic microorganisms and toxic chemicals (heavy metals, nitrates/nitrites, pesticides), with a pH between 6.5 and 8.5. This paper aims to clarify the actual quality of drinking water from various sources and correlate the experimental results with WHO/EU and Romanian (STAS) standards.

The theoretical background details physical properties (turbidity, color, taste, odor), chemical properties (pH, conductivity, dissolved oxygen), types of contaminants (biological and chemical), and main sources of pollution (agriculture, industry, urban waste, natural processes). The health risks associated with nitrates/nitrites (e.g., infant methemoglobinemia), heavy metals (lead, mercury, cadmium), and the impact of organic compounds and chlorination by-products (trihalomethanes) are reviewed.

The aim was to determine the physico-chemical composition and toxic contaminants in ten water samples, compare results with WHO/EU and STAS limits, assess potential health risks, and propose practical recommendations.

The hypotheses addressed differences between bottled, tap (filtered/unfiltered), and well water; potential exceedance of permissible limits in non-bottled samples; and variable filtration efficiency.

2. MATERIALS AND METHODS

Determinations were performed using the JBL PRO Aquatest Combi Set Plus (NH_4) according to experimental sheets, applying rapid colorimetric methods for pH, nitrates (NO_3^-), nitrites (NO_2^-), and ammonium (NH_4^+). A qualitative electrolysis test was used for assessing dissolved ions. Results were compared to WHO/EU and Romanian reference limits (STAS 1342/91).

Summary of methods:

- pH (optimal 6.5–8.5): Colorimetric measurement (pH reagent 3–10); after 5 minutes, color compared with standard scale.
- Nitrates (WHO limit ≤50 mg/L): Reagents NO_3 no.1 and no.2; 10-minute color development; comparison with scale.
- Nitrites (WHO/EU limit ≤0.1 mg/L): Rapid colorimetric method; visual reading.
- Ammonium (recommended <0.5 mg/L): NH_4 reagent; 5-minute reaction; reading against scale.
- Electrolysis (qualitative orientation): Observation of color changes indicating ionic presence (Fe^{3+}, Mn, etc.).

Table. 1 Analyzed sources (samples P1–P10):

Sample	Water Source
P1	Zizin (bottled water)
P2	Aqua Carpatica (bottled water)
P3	San Benedetto (bottled water)
P4	Bucovina (bottled water)
P5	Izvorul Minunilor (bottled water)
P6	Perla Harghita (bottled water)
P7	Tap water – filtered
P8	Tap water – unfiltered
P9	Well – 8 m
P10	Well – 50 m

3. RESULTS AND DISCUSSION

1) pH of analyzed samples:

All samples had pH values between 6.5 and 8.0, within the potability range. Bottled waters showed slightly higher stability around 7.0–7.5.

Table 2- *pH Determination*

Proba nr.	**Sursa de apă**		**Valoare pH**	**Observații**
1	Zizin		7.5	
2	Aqua Carpatica		7.5	
3	San Benedetto		7.5	
4	Bucovina		6.5-7	
5	Izvorul Minunilor		7	
6	Perla Harghita		6.5-7	
7	Apă de la filtru		6.5-7	
8	Apă de la robinet		7.5	
9	Apă fântână 8 m		8	
10	Apă fântână 50 m		8	

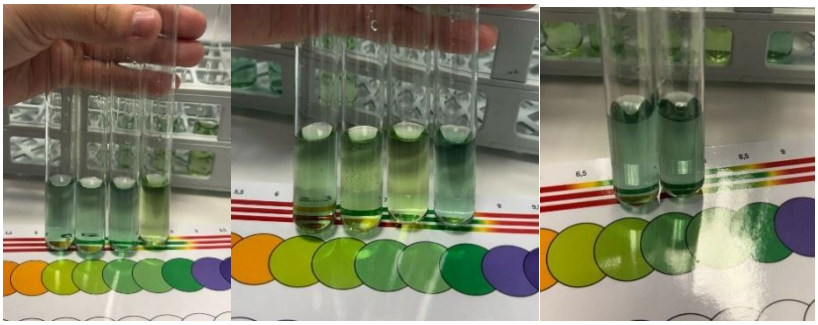

Foto 1- pH Determination

2) Nitrite content :

Bottled waters (P1–P5) showed very low nitrite levels (<0.01 mg/L).
Samples P6–P9 recorded 0.025 mg/L (below the 0.1 mg/L limit).
Sample P10 (deep well, 50 m) showed 0.8 mg/L, significantly exceeding the limit — not compliant and unfit for consumption.

Nitrates content

Table 3 – *Nitrates content*

Proba nr.	Sursa de apă	Intensitate culoare	Nitrați (mg/L)	Observații
1	Zizin	alb	<0,5	
2	Aqua Carpatica	Galben foarte deschis	1	
3	San Benedetto	Alb	<0,5	
4	Bucovina	Alb	<0,5	
5	Izvorul Minunilor	Alb	<0,5	
6	Perla Harghita	Alb	<0,5	
7	Apă de la filtru	Alb	<0,5	
8	Apă de la robinet	Alb	<0,5	
9	Apă fântână 8m	Galben deschis intens	25	
10	Apă fântână 50 m	Portocaliu	30	

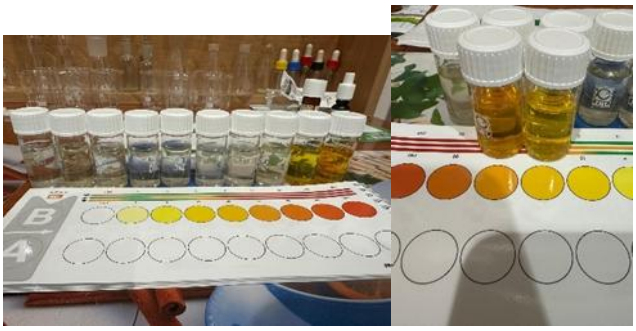

Foto 2- Results NO3; Results from Well 8m & 50m

3) Ammonium content (NH_4^+):

Most samples were below 0.05 mg/L, except San Benedetto (P3) with 0.1 mg/L, still within acceptable limits.

4) Electrolysis observations (qualitative):

- P4 (Bucovina) and P5 (Izvorul Minunilor): low mineral content.
- P6 (Perla Harghita) and P7 (filtered tap): colors suggested high iron content (oxidation).
- P8 (unfiltered tap): possible dissolved iron from pipe corrosion.
- P9–P10 (wells): green-brown colors, indicating high organic/mineral load and conductivity.

Comparison with who/eu and stas standards

Reference thresholds: pH 6.5–8.5; nitrates ≤50 mg/L; nitrites ≤0.1 mg/L; ammonium <0.5 mg/L.
All samples met the pH criterion; nitrites were within limits in P1–P9, except P10 (0.8 mg/L); ammonium was below 0.5 mg/L in all cases.

Differences between water types were clear. Bottled waters showed consistent quality and low contaminant levels, while well waters presented vulnerabilities (notably nitrites in P10), likely from agricultural or local infiltration.

Unfiltered tap water may accumulate iron from old pipes; household filters improve but do not fully remove ions.

For non-compliant sources (e.g., P10), the paper recommends sanitary isolation of wells (sealing, repair of cracks), surface leak prevention, installation of activated carbon and/or reverse osmosis filters, and periodic chemical and microbiological monitoring.
Compliance with WHO/EU/national standards remains essential for protecting public health.

Legal framework and relevant standards

The study references:

- WHO Guidelines for Drinking-Water Quality;
- EU Directive (EU) 2020/2184 on water quality for human consumption;
- Romanian acts: Government Ordinance no. 7/2023, Water Law no. 107/1996, Government Decision no. 974/2004, Ministry of Health Order no. 119/2014, and STAS 1342/91;
- National Institute of Statistics (2023) data on population water access;
- European Environment Agency reports on Europe's water resources.

4. CONCLUSIONS

Bottled waters (P1–P6) meet all tested parameters and are safe for daily consumption.
Well waters require regular monitoring, strict maintenance/isolation, and treatment solutions (activated carbon, reverse osmosis).
Filtered tap water can be a viable option if systems are properly chosen and maintained.
Simultaneous evaluation of multiple indicators (pH, NO_3^-, NO_2^-, NH_4^+, and qualitative electrolysis results) is essential for accurate potability assessment.
Recommendations must align with WHO/EU standards and national legislation, and public awareness remains a key health priority.

BIBLIOGRAPHY:

1.Berca C. – Apa şi sănătatea-apa potabilă, ape minerale, hidroterapia. Editura Ceres, București, 1994.
2.Negulescu M. și colab. – Protecția calității apelor. Editura tehnica, București, 1982.
3.Stoianovici S., Robescu D. – Procedee şi echipamente mecanice pentru tratarea şi epurarea apei. Editura Tehnica, București, 1982.
4.Teodosiu Carmen – Tehnologia apei potabile şi industriale. Editura Matrix Rom, București, 2001.
5.Rojanschi V. – Alimentarea cu apă – la punct de răscruce. Editura Ceres, București, 1983.
6.Robescu D., Stamatoiu D. – Bătălia pentru apa vie. Editura Ceres, București, 1988.
7.Robescu D., Robescu Diana – Fiabilitatea proceselor, instalațiilor şi echipamentelor de tartare şi epurare a apelor. Editura tehnica, București, 2002.
8.Rusu G., Rojanschi V. – Filtrarea în tehnica tratării şi epurării apelor. Editura tehnica, București, 1980.
9.Ordonanţa nr. 7/2023 privind calitatea apei destinate consumului uman.
10.Legea nr. 458/2002 privind calitatea apei potabile.
11.STAS 1342/91 – Calitatea apei potabile;
12.https://lege5.ro/gratuit/geztenbshe3tc/ordonanta-nr-7-2023-privind-calitatea-apei- destinate-consumului-uman
13.https://ro.wikipedia.org/wiki/Ap%C4%83
14.https://alba24.ro/atentie-de-unde-beti-apa-doar-4-izvoare-din-alba-au-apa-potabila- 109757.html
15.https://apaprod.ro/statii-de-tratare-a-apei/
16.https://ro.pinterest.com/andratroneci/apa-de-ploaie/
17.https://view.livresq.com/view/5fe88a634395010007d37084/
18.https://republica.ro/ai-bea-apa-reciclata
19.https://mindcraftstories.ro/mediu/eco-impact-risc-de-criza-a-apei-potabile-la-nivel-mondial/?utm_source=chatgpt.com
20.https://www.euractiv.ro/eu-elections-2019/agentia-europeana-de-mediu-progrese- invizibile-in-domeniul-protejarii-apelor-in-europa-69084

GREEN SYNTHESIS OF CONDUCTIVE HYDROGELS INCORPORATING SILVER NANOPARTICLES FROM *CURCUMA LONGA* FOR TRANSDERMAL DICLOFENAC DELIVERY

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DOI: https://doi.org/10.66793/titum19proceeding20

Abstract

This work reports the green synthesis and characterization of a conductive hydrogel incorporating silver nanoparticles (AgNPs) biosynthesized from Curcuma longa extract for the transdermal delivery of diclofenac. The hydrogel matrix was based on poly(vinyl alcohol) (PVA) and poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate) (PEDOT:PSS), ensuring mechanical stability and electrical conductivity. The biosynthesized AgNPs exhibited a characteristic plasmon resonance around 430 nm, confirming their formation and stability. FTIR and UV–Vis spectroscopy confirmed the interaction between PVA, PEDOT:PSS, and AgNPs, while SEM micrographs revealed a porous, interconnected morphology suitable for drug diffusion. Swelling and rheological analyses demonstrated high water retention and viscoelastic stability, while diclofenac release studies showed a sustained release profile. The resulting hybrid system represents a biocompatible, electrically responsive platform for advanced transdermal drug delivery applications.

1. INTRODUCTION

Hydrogels are three-dimensional polymeric networks capable of absorbing large quantities of water, mimicking the extracellular matrix of biological tissues. Their biocompatibility and ability to respond to external stimuli such as pH, temperature, and electrical fields make them suitable for a wide range of biomedical applications, including drug delivery, wound healing, and biosensing. Conductive hydrogels (CHs) combine the hydration properties of conventional hydrogels with the electrical conductivity of conjugated polymers, such as polyaniline (PANI) or poly(3,4-ethylenedioxythiophene):poly(styrenesulfonate) (PEDOT:PSS). These hybrid materials enable electro-responsive drug release and facilitate communication with biological tissues. The incorporation of metallic nanoparticles such as silver (AgNPs) can further enhance the functional properties of hydrogels. Green synthesis routes using plant extracts as reducing and stabilizing agents have emerged as sustainable alternatives to conventional chemical synthesis. Curcuma longa (turmeric) contains curcuminoids and phenolic compounds capable of reducing Ag^{+} ions to Ag^{0} nanoparticles, while simultaneously capping and stabilizing them. These AgNPs provide antibacterial and anti-inflammatory properties, making them ideal for topical and transdermal systems. Diclofenac, a non-steroidal anti-inflammatory drug (NSAID), was chosen as the model compound to evaluate controlled drug release from the hydrogel.

2. EXPERIMENTAL DETAILS

2.1 Materials

Poly(vinyl alcohol) (PVA, 98–99% hydrolyzed), PEDOT:PSS (1.3 wt% aqueous dispersion), and diclofenac sodium were obtained from Sigma-Aldrich. Fresh rhizomes of Curcuma longa were used for the biosynthesis of AgNPs. All aqueous solutions were prepared with deionized water.

2.2 Green synthesis of silver nanoparticles (AgNPs)

An aqueous extract of Curcuma longa was prepared by boiling 10 g of finely ground turmeric powder in 100 mL of distilled water for 10 min, followed by filtration through Whatman No. 1 paper. To 80 mL of 1 mM $AgNO_3$ solution maintained at 60 °C under stirring, 5 mL of the turmeric extract was added dropwise. The color change from pale yellow to brown indicated the formation of silver nanoparticles. The colloidal suspension was centrifuged at 12 000 rpm for 15 min and redispersed in water for further use.

2.3 Preparation of PVA/PEDOT:PSS-AgNP hydrogel loaded with diclofenac

A 10 wt% PVA solution was prepared by dissolving PVA in distilled water at 90 °C with constant stirring. After cooling to 40 °C, 5 wt% PEDOT:PSS dispersion and the biosynthesized AgNPs (2 mL, $A_{430} \approx 1.0$) were added and homogenized. Diclofenac sodium (0.1 g per 10 mL of polymer solution) was incorporated, and the mixture was cast into Petri dishes. The hydrogel was formed by three freeze–thaw cycles (–20 °C/4 h followed by thawing at 25 °C/1 h).

2.4 Characterization techniques

UV–Vis spectra were recorded using a Hitachi U-0080D spectrophotometer in the range 200–800 nm. Fourier transform infrared (FTIR) spectra were obtained with a Bruker Tensor 27 spectrometer using ATR mode. Surface morphology was analyzed by scanning electron microscopy (SEM, Nova NanoSEM 630). Swelling experiments were performed by immersing pre-weighed hydrogel discs in phosphate-buffered saline (PBS, pH 7.4) at 37 °C. The swelling ratio was calculated as $(W_t - W_0)/W_0$. Drug release studies were performed in PBS (pH 7.4) at 37 °C and absorbance at 276 nm was monitored spectrophotometrically.

3. RESULTS AND DISCUSSION

3.1 UV–Vis analysis

To confirm the formation of silver nanoparticles (AgNPs) synthesized using turmeric extract, UV–Vis absorption spectra were recorded for several samples during synthesis(Figure 1). The obtained spectra exhibited a characteristic surface plasmon resonance (LSPR) band of AgNPs in the 420–440 nm region, confirming their successful formation. A progressive increase in absorption intensity was observed from the light pink to the dark red sample, indicating a higher concentration of nanoparticles. This enhancement in absorption can be correlated with variations in the reaction parameters (extract:$AgNO_3$ ratio = 1:6, reaction time = 45 min, temperature = 60 °C, etc.), suggesting different efficiencies in silver ion reduction and nanoparticle stabilization under distinct experimental conditions.

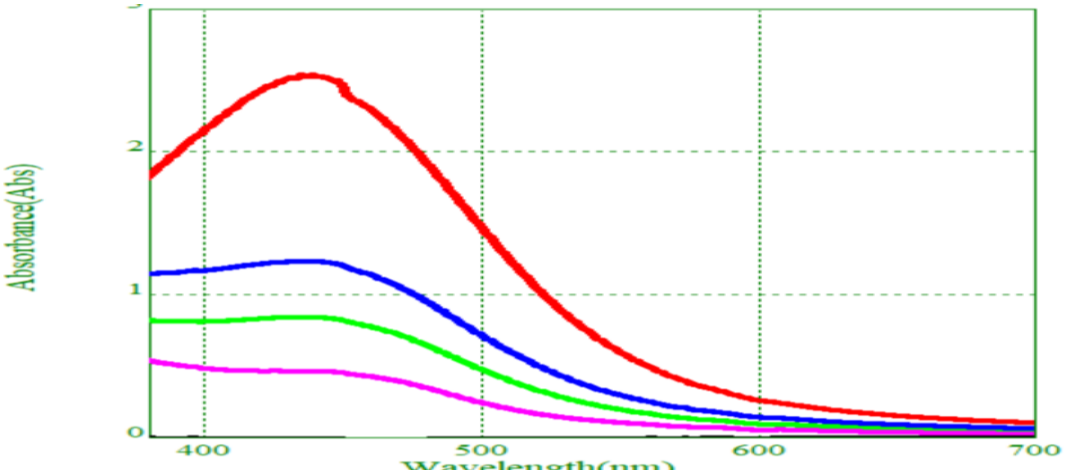


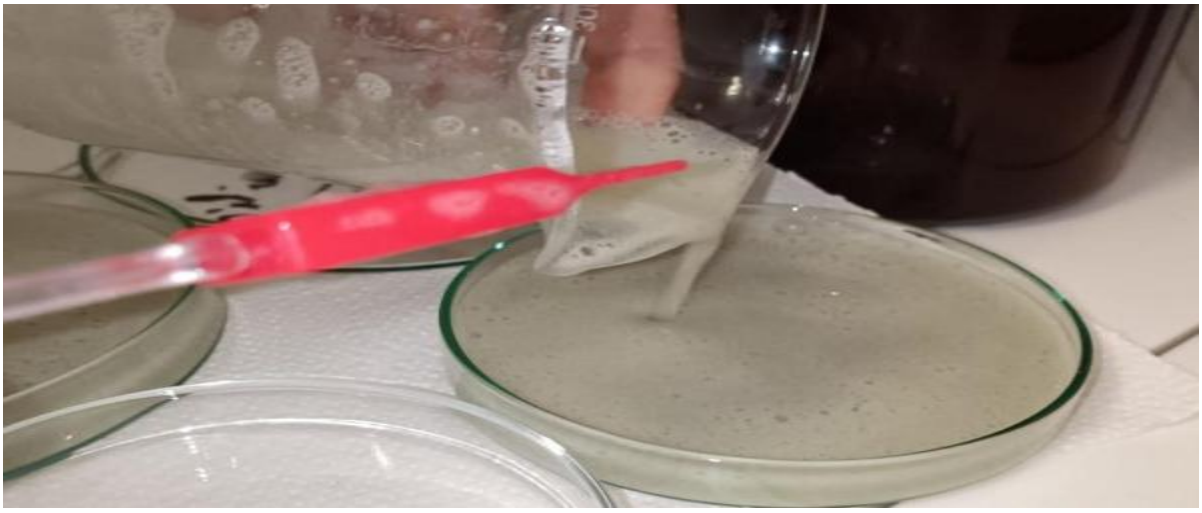
Figure.1 UV–Vis absorption spectra were recorded for several samples during green synthesis

The visual appearance of the reaction mixtures provided qualitative evidence of nanoparticle formation and stability. In Figure 2, five representative samples (1–5) are shown, displaying a clear color shift from pale yellow to dark orange. These color variations reflect both particle size and nanoparticle concentration, as supported by previous reports. The more intensely colored samples corresponded to higher absorbance values in the UV–Vis spectra (Figure 1), confirming a more efficient synthesis process.

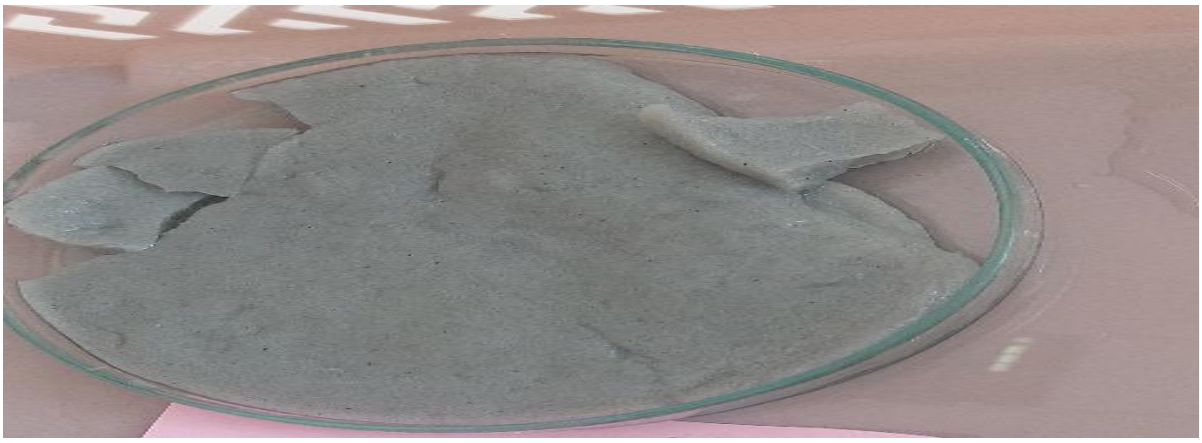
Figure 2. Samples numbered 1–5 obtained after the reaction between turmeric extract and $AgNO_3$. The color intensity varies depending on the efficiency of Ag nanoparticle synthesis.

3.2 Preparation and Characterization of PEDOT–PVA–Chitosan–AgNP–Diclofenac Composite Hydrogel

To obtain a functional material with biomedical potential, a composite hydrogel based on PEDOT, PVA, chitosan (CS), silver nanoparticles synthesized from turmeric extract (AgNPs), and sodium diclofenac was prepared using a thermal gelation method followed by casting in Petri dishes. The gelation process and the physical appearance of the samples were visually monitored, as shown in Figure 3 (A–B).



A.



B

Figure 3. Visual representation of the PEDOT–PVA–CS–AgNP–diclofenac hydrogel formation process.

(A) Casting of the homogeneous hydrogel mixture into Petri dishes during preparation.

(B) Appearance of the formed composite hydrogel after gelation and solidification.

Initially, a homogeneous suspension containing the polymers PEDOT (poly(3,4-ethylenedioxythiophene)), PVA (polyvinyl alcohol), and chitosan was prepared in a suitable solvent medium to allow uniform dissolution and mixing. Chitosan, a biopolymer derived from chitin, provides antimicrobial properties and biocompatibility, while PVA acts as a stabilizing and crosslinking agent that enhances mechanical integrity. PEDOT contributes electronic conductivity and electroactivity to the hybrid hydrogel. The reaction mixture was poured into Petri dishes (Figure 3A–B) and allowed to gel under controlled temperature conditions. Gelation was driven by intermolecular interactions among the functional groups of the polymers and physical–chemical crosslinking induced by thermal treatment. The silver nanoparticles were previously synthesized via a green route using turmeric extract, which acts as both a reducing and stabilizing agent. The presence of curcumin contributes to colloidal stability and introduces antioxidant functionality into the system.

The synthesized AgNPs were subsequently incorporated into the polymeric matrix along with sodium diclofenac, a non-steroidal anti-inflammatory drug (NSAID), to produce a composite hydrogel with enhanced therapeutic potential. Upon crosslinking, the hydrogel gradually hardened, forming a self-supporting structure. Surface contraction and minor cracking were observed as water evaporated, indicating network consolidation. The matured hydrogel exhibited satisfactory elasticity, cohesion, and water-retention capacity, features essential for biomedical or pharmaceutical applications.

The final gelled material was portioned into compact discs or small fragments for further use, depending on the intended application. The measured specific conductivity of the composite hydrogel was 2.05 mS cm^{-1} at 25 °C, indicating good ionic transport and suitability for use in controlled drug-delivery systems or electrochemical biosensors.

3.2 FTIR Characterization of Synthesized PEDOT and Composite Hydrogel

The FTIR spectra of the PVA/PEDOT:PSS–AgNP hydrogel showed broad O–H stretching at 3300 cm^{-1}, C=O bands around 1640 cm^{-1}, and peaks corresponding to sulfonate groups from PSS at 1030 cm^{-1}. The shift in these peaks compared to pure PVA confirmed hydrogen bonding and electrostatic interactions among the components. The presence of diclofenac was identified through characteristic C–Cl and aromatic C=C bands at 748 cm^{-1} and 1500 cm^{-1}.

The PEDOT obtained through this method is non-processable (insoluble) but can be dispersed in water using a surfactant such as sodium dodecyl sulfate (SDS) for various applications.

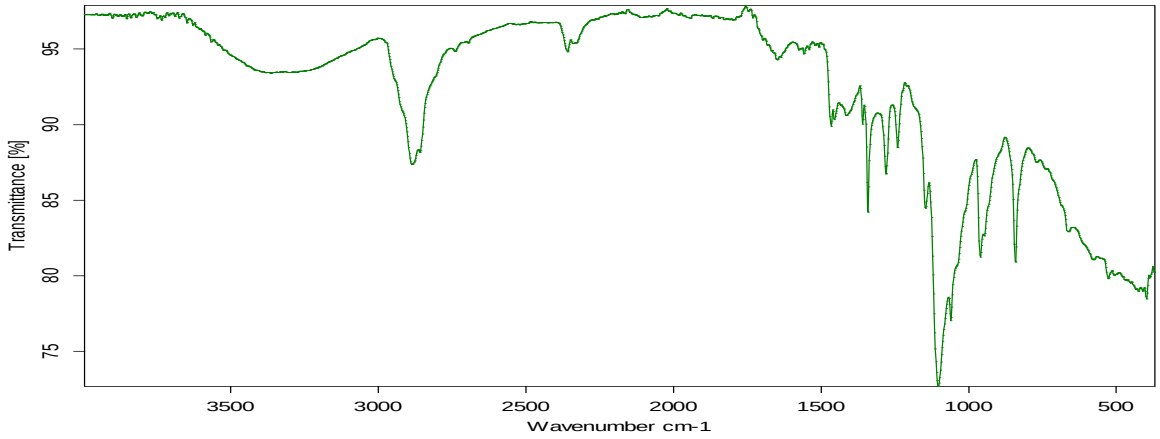


Figure 4. FTIR spectrum of the PEDOT/PVA/chitosan/AgNP composite hydrogel loaded with turmeric and diclofenac. The spectrum shows characteristic absorption bands corresponding to functional groups O–H, N–H, C=O, and C–O–C, indicating chemical interactions between the matrix components and the active

The FTIR spectrum of the synthesized PEDOT polymer displays characteristic absorption bands confirming the formation of the conjugated polymer structure. The broad band observed at 1510–1550 cm^{-1} corresponds to the C=C stretching vibrations of the thiophene ring, indicating the presence of conjugated aromatic segments. In the region of 1300–1350 cm^{-1}, a distinct band is attributed to inter-ring C–C stretching, typical of the rigid and planar PEDOT backbone. Another significant band appearing in the 1130–1200 cm^{-1} region is assigned to C–O–C stretching vibrations, characteristic of the ethylenedioxy groups within the EDOT unit. The presence of this band confirms successful polymerization from the EDOT monomer.
A sharp band located near 980–990 cm^{-1} corresponds to C–S–C deformation of the thiophene ring, while the signal around 840–870 cm^{-1} is associated with C–H out-of-plane vibrations. A weak band between 700–750 cm^{-1} represents the ring deformation mode of the thiophene unit. These spectral features confirm the formation of doped PEDOT with possible contributions from polaronic and bipolaronic states, as later verified by UV–Vis spectroscopy. Therefore, FTIR analysis confirms the successful synthesis of PEDOT with preservation of its conjugated structure, essential for achieving the desired electrical properties.

3.3 SEM morphology

SEM micrographs revealed a porous, interconnected structure with uniformly distributed silver nanoparticles embedded within the polymeric network. The pore size (20–50 µm) facilitates efficient water absorption and drug diffusion. The incorporation of PEDOT:PSS imparted a smooth, conductive texture (Figure 5).

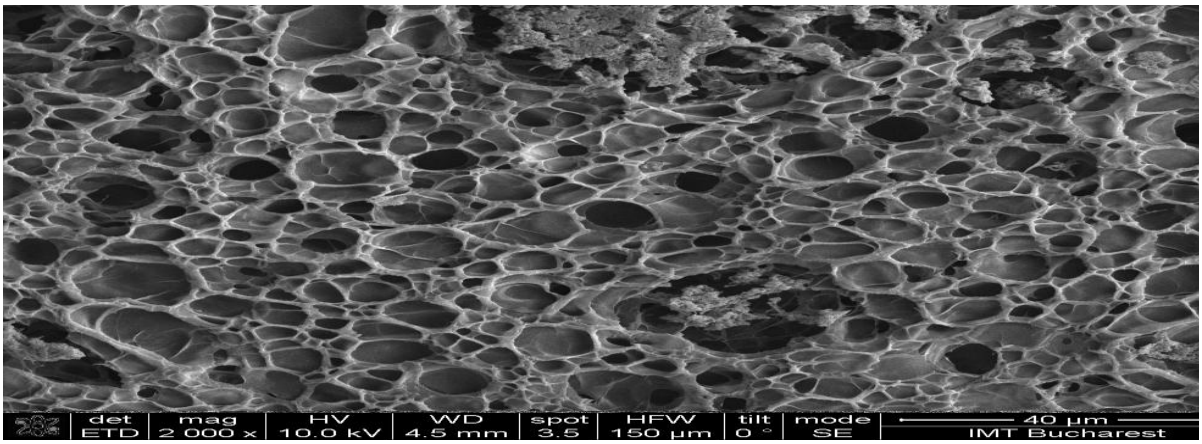


Figure 5. SEM of PEDOT/PVA/chitosan/AgNP composite hydrogel

3.4 Swelling behavior

The swelling ratio reached equilibrium after 4 h, with a maximum of 420%, indicating high hydrophilicity. Rheological analysis demonstrated a dominant elastic response ($G' > G''$), suggesting strong physical cross-linking and mechanical stability suitable for skin application.

3.5 Diclofenac release study

The release profile showed an initial burst within the first hour, followed by sustained diffusion over 24 h, consistent with Fickian diffusion kinetics (Figure 6). The presence of AgNPs slightly slowed the release due to reduced mesh size and possible drug–nanoparticle interactions. This indicates the potential for prolonged anti-inflammatory action in transdermal systems.

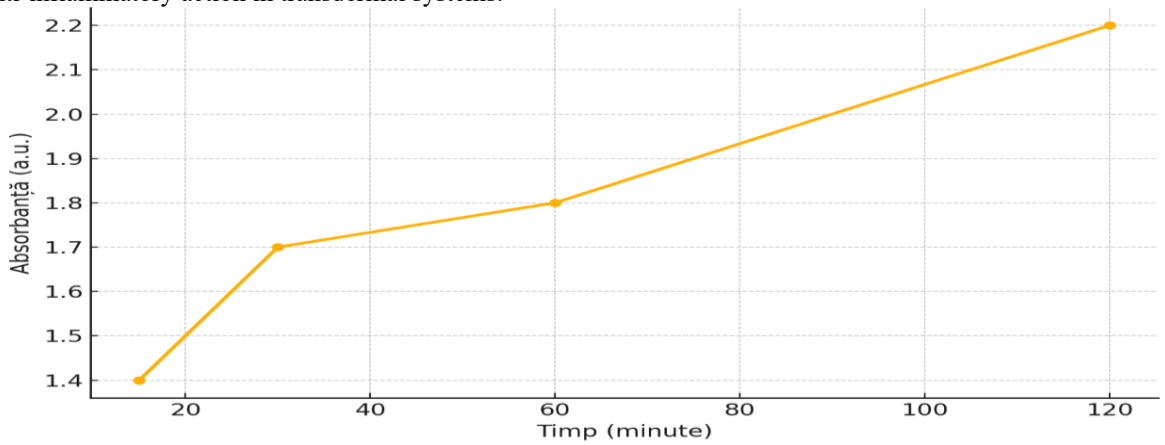


Figure 6. Diclofenac release profile from the PEDOT/PVA/chitosan/AgNP composite hydrogel over time. The progressive increase in absorbance at 276 nm indicates sustained drug diffusion from the polymeric matrix up to 120 minutes.

4. CONCLUSIONS

A conductive PVA/PEDOT:PSS hydrogel incorporating green-synthesized AgNPs from Curcuma longa was successfully prepared and characterized. The hybrid hydrogel exhibited favorable structural, swelling, and mechanical properties, along with sustained diclofenac release. Its combination of biocompatibility, conductivity, and antibacterial potential highlights its suitability for next-generation transdermal delivery platforms and wearable therapeutic systems.

REFERENCES

[1] Raveendran, P., Fu, J., & Wallen, S. L. (2003). Completely green synthesis and stabilization of metal nanoparticles. J. Am. Chem. Soc.

[2] Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). A review on plants extract mediated synthesis of silver nanoparticles. J. Adv. Res.

[3] Chen, S. et al. (2024). Conductive hydrogels for biomedical applications. Biosensors.

[4] Gu, D. et al. (2016). Structural and mechanical properties of PVA/PEDOT:PSS hydrogels. Polymers.

POLYPHENOL (APFC) CONTENT IN CERTAIN PLANT EXTRACTS CORRELATED WITH ANTITUMOR THERAPEUTIC ACTIVITY

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DOI: https://doi.org/10.66793/titum19proceeding21

Abstract

Phytotherapy is widely used to treat and prevent various medical conditions and is well known among the general population. Interest in natural compounds with therapeutic potential has increased significantly, as they represent a rich source of bioactive substances with diverse pharmacological properties. In this article, we will analyze the composition of polyphenols and the biological roles of some plants, highlighting their importance in phytotherapy and nutrition. Comparing three plants, it is observed that each has a unique polyphenol composition: plantain contains predominantly flavonoids and caffeic acid, rhubarb is rich in anthraquinones and phenolic acids, and echinacea contains chicoric acid and echinacoside. Thus, the three plants complement each other and can be associated in natural therapies aimed at immune protection and cell regeneration.

1. INTRODUCTION

Phytochemistry, the main branch of Pharmacognosy, has as its basic objective the isolation of phytochemical compounds contained in various plant and animal raw materials, through the use of different technological processes, and subsequently the identification and dosage of these compounds through physicochemical methods frequently used in specific laboratories [1, 2].

APFC (Antioxidant Polyphenolic Functional Compounds) polyphenols represent a complex category of natural compounds present in the plant kingdom. They are recognized for multiple beneficial effects on the human body: antioxidant properties (neutralize free radicals and protect cells), anti-inflammatory effects (reduce chronic inflammation), anticancer effects (inhibit tumor cell proliferation), cardiovascular protection (improve blood vessel function), and neuroprotective effects (help maintain brain health) [3].

APFC polyphenols are found in numerous medicinal plants, including plantain, rhubarb, and echinacea. Each plant contains a specific profile of polyphenols responsible for its therapeutic effects. In this article, we will analyze these plants' polyphenol composition and biological roles, highlighting their importance in phytotherapy and nutrition [4].

Plantain is one of the most widely used medicinal plants in Romania's wild flora. It contains a wide range of polyphenols, including caffeic acid, ferulic acid, chlorogenic acid, verbascoside (acteoside), luteolin, and apigenin. Plantain leaves are also rich in tannins, which contribute to their astringent and antibacterial effects [5].

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Rhubarb is a perennial plant known for its laxative, hepatoprotective, and antitumor properties. Its rich content of polyphenols explains many of its therapeutic actions. Among the most important compounds are gallic acid, caffeic acid, and catechins [6].

Echinacea is one of the most popular immunostimulating plants. It contains numerous polyphenolic compounds, the most important of which are chicoric acid, caffeic acid, ferulic acid, echinacoside, and kaempferol. Of these, chicoric acid is considered the primary phytochemical marker of the plant [7].

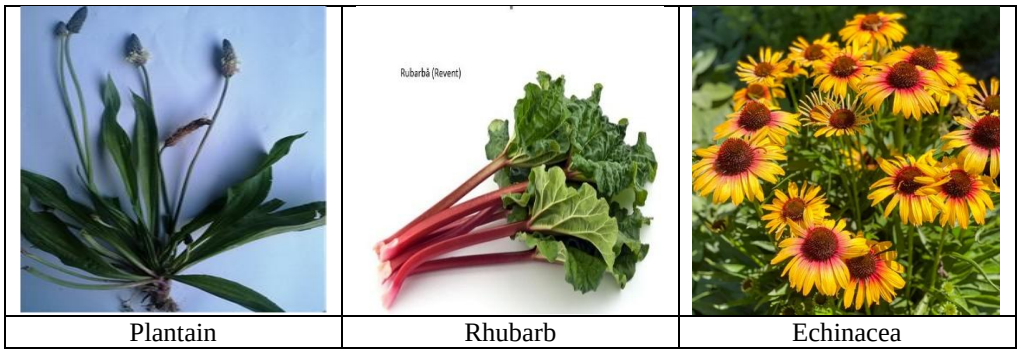
2. EXPERIMENTAL DETAILS

Compounds isolated from plant materials are analyzed through qualitative and quantitative methods to determine these products' identity, purity, and quality with recognized therapeutic potential. We identified and characterized the bioactive compounds in plantain, rhubarb, and echinacea extracts using advanced analytical techniques, exploring how they influence therapeutic activity.

Plantain is a herbaceous plant, widely distributed throughout the world. It is known for its ability to survive in diverse environmental conditions and its rich chemical composition, which includes a variety of bioactive compounds, such as polyphenols, flavonoids, iridoids, and terpene compounds. Plantain is a valuable plant appreciated for its multiple benefits to the body. It contains a variety of bioactive compounds, including flavonoids, alkaloids, terpenoids, phenolic compounds (derivatives of caffeic acid), iridoid glycosides, fatty acids, polysaccharides, and vitamins (**Figure 1**).

Rhubarb is one of the oldest, most commonly used, and important medicinal plants in Chinese medicine. Modern research on rhubarb has clarified its efficacy, phytochemical compounds, and mechanisms of action in a more scientific and rigorous way. The main chemical constituents of rhubarb include anthraquinones, anthrones, stilbenes, tannins, and polysaccharides (**Figure 1**).

Echinacea is a medicinal plant native to North America. It has been used for centuries to strengthen the immune system and prevent colds and diseases. Echinacea is one of the most important modern medicinal plants, with remarkable properties in stimulating immunity and preventing diseases. Used correctly, it helps maintain the body's health and protects against infections (**Figure 1**).



Plantain	Rhubarb	Echinacea

Figure 1. Medicinal plants

2.1. Obtaining plantain, rhubarb and echinacea extracts in the laboratory

Extracts derived from plantain, rhubarb, and echinacea were obtained through a systematic series of processes utilizing hydroalcoholic or aqueous solvents. The extracts generated are characterized by their hydroalcoholic or aqueous nature.

The isolation of water-soluble active principles from the studied plant products is carried out by aqueous extraction. Ethyl alcohol is used in different concentrations (30-70%) to isolate compounds soluble in organic solvents. In the laboratory experiments, alcoholic extraction was used to obtain tinctures, hydroalcoholic extracts by various methods, such as maceration, percolation, and reflux extraction. In the laboratory, hydroalcoholic extracts were obtained from plantain, rhubarb, and echinacea powder by the reflux extraction process, with 30%, 50% and 70% alcohol and a tincture, obtained by cold maceration with ethyl alcohol of 50-70% concentration.

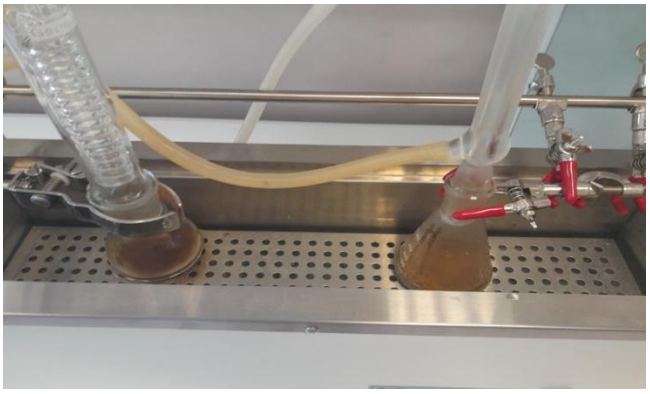


Figure 2. Reflux extraction process

These laboratory-obtained extracts were used to identify and study the dosage of active compounds from plantain, rhubarb, and echinacea.

2.2. Physico-chemical characteristics of the raw material of plantain, rhubarb, and echinacea

According to the data presented in the monograph of the European Pharmacopoeia, the current edition for the plant products plantain, rhubarb, and echinacea, the pharmacognostic analysis provides for a series of qualitative and quantitative analysis methods. The identification methods are based on color or precipitation reactions with the necessary reagents. High-performance instrumental techniques such as UV-Vis spectrophotometry and flame atomic absorption spectrometry are used for the dosages.

2.3. Identification of flavone derivatives

The identification of flavone derivatives present in plant products such as plantain, rhubarb, and echinacea was carried out in the laboratory based on the color reaction that occurs in the presence of aluminum chloride and sodium acetate. In this case, a yellow color is observed, characteristic of the presence of flavone derivatives in the sample to be analyzed.

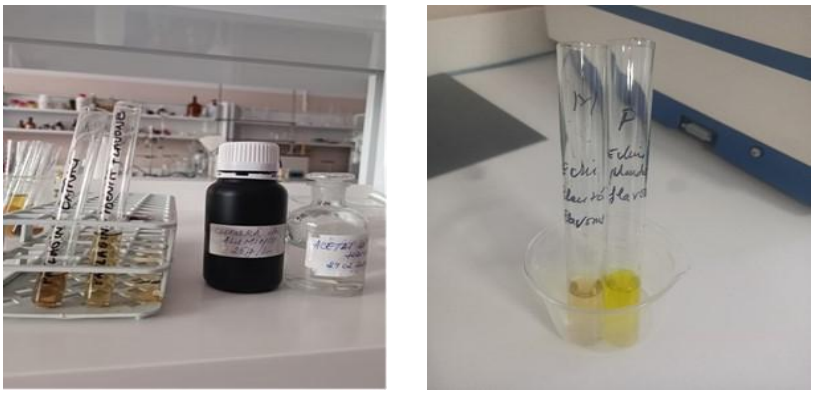


Figure 3. Identification of flavones from the extracts

2.4. Identification of polyphenolcarboxylic acids

From the plant products plantain, rhubarb, and echinacea, to identify phytochemical compounds with antioxidant properties from the category of polyphenolcarboxylic acids, the color reaction that occurs in the presence of Folin

Ciocâlteu reagent and sodium carbonate solution was used. In this case, the appearance of a blue-green color is characteristic of the presence of these compounds.

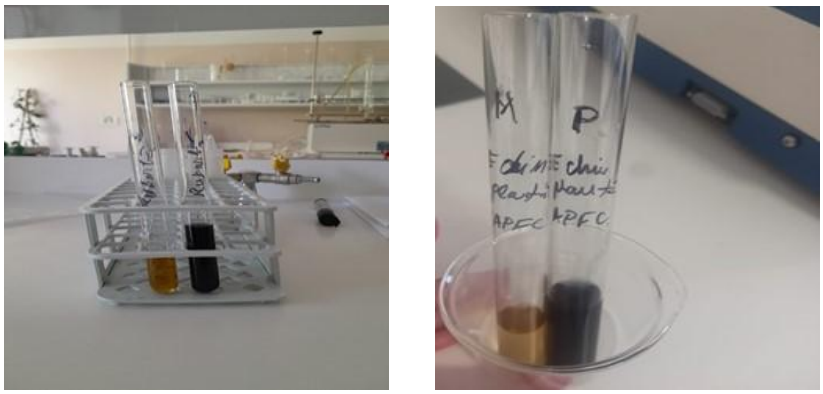


Figure 4. Identification of polyphenolcarboxylic acids

2.5. Determination of total polyphenol content expressed in gallic acid equivalent and caffeic acid equivalent in plantain, rhubarb and echinacea

The total polyphenols expressed in gallic acid equivalent and caffeic acid equivalent was determined using UV-Vis spectrophotometry. Samples of the plant product, plantain, rhubarb and echinacea powder and tincture prepared in the laboratory by maceration in 70% ethyl alcohol, and a series of hydroalcoholic extracts obtained by reflux extraction with 30%, 50% and 70% ethyl alcohol, as well as an aqueous extract obtained by reflux extraction, were analyzed.

Figure 5. Determination of total polyphenols by UV-Vis spectrophotometry

To determine the total polyphenol content, 5 mL of diluted Folin–Ciocalteu reagent is added to a volume of 1 mL of test solution, homogenized by stirring. After an interval of 3–5 minutes, it is completed with 4 mL of sodium carbonate solution (7.5%). The mixture obtained is left to stand for one hour, a period necessary for developing the specific color of the phenolic complex. Subsequently, the spectrophotometric absorption of the solution is determined at a wavelength of 765 nm (for gallic acid equivalent) and 748 nm (for caffeic acid equivalent). As a control (blank), a sample prepared identically to the one to be analyzed is used, in which 9 mL of purified water, without Folin–Ciocalteu reagent, is added to 1 mL of extract. Using the same spectrophotometric equipment, the total polyphenol concentration is calculated based on the calibration curve obtained for gallic acid (at 765 nm) and caffeic acid (at 748 nm). The results are expressed in µg/mL, and the values obtained are presented in the Table, corresponding to total polyphenols expressed in gallic acid equivalent and caffeic acid equivalent.

Table 1. Total polyphenol content expressed in gallic acid and caffeic acid equivalents from extract samples

Plant product	Sample name	Total polyphenol content - gallic acid, [g/100g]	Total polyphenol content - caffeic acid, [g/100g]
Rhubarb	Aqueous extract	0,04	0,036
	30% ethyl alcohol extract	3,5	3,2
	70% ethyl alcohol extract	4,9	4,4
	Tincture	4,6	4,3
Echinacea	Aqueous extract	0,21	0,19
	30% ethyl alcohol extract	0,28	0,24
	70% ethyl alcohol extract	0,56	0,52
	Tincture	0,52	0,48
Plantain	Aqueous extract	0,18	0,10
	30% ethyl alcohol extract	2,6	2,2
	70% ethyl alcohol extract	3,1	2,9
	Tincture	3,0	2,7

3. ANTITUMOR EFFECTS OF PLANT PRODUCTS CONTAINING POLYPHENOLS

Polyphenolic compounds exhibit multiple mechanisms of antitumor action, acting at different stages of cancer development - initiation, promotion, and progression. Their effects include:

a) inhibition of cell proliferation (certain flavonoids, such as quercetin, apigenin, and luteolin, interfere with cell cycle progression by inducing G1 or G2/M arrest, reducing tumor cell proliferation)

b) induction of apoptosis (phenolic acids and anthraquinones activate intrinsic apoptotic pathways, increasing the expression of pro-apoptotic proteins (Bax, caspase-3) and reducing anti-apoptotic proteins (Bcl-2)

c) suppression of angiogenesis and metastasis

d) epigenetic modulation

e) immunomodulatory effects (plant extracts enhance the activity of macrophages and natural killer (NK) cells, increasing the ability of the immune system to identify and eliminate malignant cells)

Polyphenolic compounds represent a valuable category of natural antioxidants with demonstrated antitumoral potential [8]. Their multifactorial mechanisms-from free radical scavenging to apoptosis induction and immune modulation-make them promising candidates for preventive and adjuvant cancer therapy. The continuous study of polyphenols as individual molecules and as part of complex plant extracts offers new directions in developing natural and biocompatible anticancer agents [9].

4. CONCLUSIONS

Polyphenols (APFC) are an important group of natural compounds that play an essential role in protecting the human body. Regular consumption of foods rich in polyphenols contributes to the prevention of chronic diseases,

strengthening the immune system, and maintaining cellular youth. These compounds demonstrate that nature provides valuable resources for human health, and a balanced plant-based diet is the key to longevity.

Polyphenols in plantain, rhubarb, and echinacea play an essential role in the therapeutic action of these plants. Through their antioxidant, anti-inflammatory, and immunostimulatory effects, these compounds contribute to maintaining the physiological balance of the body and preventing degenerative diseases. Controlled consumption of these plants, in teas, extracts, or supplements, can represent a valuable natural source of antioxidants.

REFERENCES

[1] Popescu, M., Compuși bioactivi din plante și rolul lor în sănătate. Editura Ceres, **2015**.
[2] Popescu, M. Plante medicinale și compuși bioactivi, Editura Ceres, **2018**.
[3] Mithul Aravind S., Wichienchot S., Tsao R., Ramakrishnan S., Chakkaravarthi S. Role of dietary polyphenols on gut microbiota, their metabolites and health benefits, *Food Research International*, 142, 110189, **2021**.
[4] Lippolis T., Cofano M., Caponio G. R., De Nunzio V., Notarnicola M. Bioaccessibility and Bioavailability of Diet Polyphenols and Their Modulation of Gut Microbiota. *International Journal of Molecular Sciences*, 24(4), 3813, **2023**.
[5] Michalak M., Plant Extracts as Skin Care and Therapeutic Agents. *International journal of molecular sciences*, *24*(20), 15444, **2023**
[6] Mendonça R.D., Carvalho N.C., Martin-Moreno J.M., Pimenta A.M., Lopes A.C.S., Martinez-Gonzalez M.A., Gea A., Bes-Rastrollo M. Total polyphenol intake, polyphenol subtypes and incidence of cardiovascular disease: The SUN cohort study, *Nutrition, Metabolism and Cardiovascular Diseases*, 29(1), 69 - 78, **2019**.
[7] Scalbert, A. & Williamson, G., Dietary intake and bioavailability of polyphenols, *The Journal of Nutrition*, **2000**.
[8] Wilczyńska A., Żak N. Polyphenols as the Main Compounds Influencing the Antioxidant Effect of Honey-A Review. *International Journal of Molecular Sciences*, 25(19), 10606, **2024**.
[9] Ciupei D., Colişar A., Leopold L., Stănilă A., Diaconeasa Z. M. Polyphenols: From Classification to Therapeutic Potential and Bioavailability. *Foods*, 13(24), 4131, **2024**.

EVALUATION OF EXTRACTION TECHNIQUES FOR ACTIVE PRINCIPLES FROM *Lavandula angustifolia* And *Rosmarinus officinalis*

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DOI: https://doi.org/10.66793/titum19proceeding22

Abstract

This paper compares some extraction methods applied to Lavandula angustifolia and Rosmarinus officinalis: cold and hot maceration, reflux extraction and Soxhlet extraction. The obtained extracts were evaluated in terms of yield and physical characteristics, emphasizing the influence of extraction technique on the recovery and quality of bioactive compounds. Hot maceration and Soxhlet extraction resulted in more concentrated extracts, characterized by increased density and refractive index, due to the enhanced release of lipophilic constituents. Cold maceration preserved thermosensitive compounds but provided lower extraction efficiency. A gradual acidification of lavender aqueous extracts was observed over time, attributed to the progressive release of phenolic components.

1. INTRODUCTION

Medicinal plants have been used for thousands of years for therapeutic purposes due to their rich content of bioactive compounds that provide numerous health benefits [1].

Today, their use in the pharmaceutical, cosmetic and food industries continues to expand, driven by the increasing demand for natural and safe products [2].

Among the most commonly used medicinal plants, *Lavandula angustifolia* (lavender) and *Rosmarinus officinalis* (rosemary) are well known for their anti-inflammatory, antioxidant, antimicrobial, wound-healing and soothing properties. Lavender is especially valued for its relaxing, antimicrobial and anti-inflammatory effects, widely applied in aromatherapy and dermatological formulations [3]. Rosemary, another valuable medicinal plant, exhibits strong antioxidant and anti-inflammatory activities, being used to improve skin health, digestion and blood circulation [4].

To fully harness their therapeutic potential, efficient extraction of active compounds – such as essential oils, flavonoids, phenolics and other bioactives – is essential, as the extraction process strongly depends on factors such as temperature, solvent type and extraction time [5].

2. EXPERIMENTAL DETAILS

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Three extraction methods were used to obtain active compounds from *Lavandula angustifolia* and *Rosmarinus officinalis*: cold and hot maceration, reflux extraction and Soxhlet extraction.
Cold maceration is a static process governed by slow diffusion and passive equilibrium, with a risk of incomplete extraction and low yield unless the extraction time is extended or combined with agitation.
Reflux extraction represents a balanced system between diffusion and constant temperature, being a versatile and efficient method for medicinal plants containing thermostable compounds.
Soxhlet extraction is an active process, thermodynamically controlled through high temperature, continuous solvent renewal, and repeated extraction cycles. This technique provides optimal yield in a relatively short time, although it involves a higher energy consumption.

2.1 Materials and methods

The refractive index (RI) and relative density shall be determined in accordance with the Romanian Pharmacopoeia [6].
The refractive index is determined with the refractometer, and the relative density with the pycnometer.
The pH is measured with an calibrated pH-meter (Consort model) and viscosity is measured with a viscometer (FungiLab model).

2.2. Synthesis procedures

For cold maceration, 20 g of each dried plant were moistened with a small amount of ethanol and mixed with 100 mL of vegetable oil. The container was sealed and shaken to remove air bubbles, then kept at room temperature for the required maceration period. After completion, the oily extract was strained through gauze and, if necessary, filtered again to obtain a clear extract. The final product was transferred into a dark container to protect sensitive compounds from light and properly labelled.
For hot maceration, the weighted plant (lavender and rosemary) was placed in a heat-resistant container with vegetable oil and heated in a water bath at 40-50ºC for 2-4 hours, with occasional stirring to prevent overheating. After extraction, the oil was strained though gauze, and filtered if necessary to obtain a clear extract. The oily extracts were transferred into amber glass containers, labelled and stored in a dry, dark place. A few drops of vitamin E were added as an antioxidant to improve storage stability.
Dried lavender (10 g) was mixed with distilled water (100 mL) in a round-bottom flask and heated in a water bath under reflux for 60 minutes. The condensed vapours continuously returned over the plant material, ensuring efficient extraction without solvent loss. After cooling, the extract was filtered and stored for further analysis.

2.3. Yield determination

The extraction yield was calculated in order to evaluate the efficiency of each extraction technique. After filtration, a measured volume of lavender extract was evaporated to dryness under controlled conditions to determine the mass of recovered bioactive fraction.
The extraction yield (%) was calculated according to the following formula:

$$\eta(\%) = \frac{m_{dry\ extract}}{m_{dried\ plant}} x100$$

3. RESULTS AND DISCUSSIONS

Following the experiments performed, significant differences were observed between the extraction methods analysed in terms of physicochemical parameters.

3.1. Characterization of physico-chemical properties

Table 1. Cold maceration

Oil extract	Density [g/cm^3]	Refractive index	Viscosity [cP]	pH
Lavender	0.92	1.470	99.22	6.5
Rosemary	0.93	1.480	191.3	6.0-6.5

Table 2. Hot maceration

Oil extract	Density [g/cm^3]	Refractive index	Viscosity [cP]	pH
Lavender	0.93	1.769	140	6.5
Rosemary	0.94	1.470	136.3	6.5

The extraction yield (%) calculated for lavender extract:

$$\eta(\%) = \frac{7.55}{8.607} x100$$

Where:

Crucible mass (empty): 4.435 g
Mass of lavender (dried plant): 8.607 g
Mass of dry extract residue: 7.55 g
The calculated extraction yield for lavender was: $\eta = 87.72\%$

The extraction yield (%) calculated for rosemary extract:

$$\eta(\%) = \frac{6.38}{8.450} x100$$

Where:
Crucible mass (empty): 4.462 g
Mass of rosemary (dried plant): 8.450 g
Mass of dry extract residue: 6..38 g
The calculated extraction yield for rosemary was: $\eta = 75.47\%$

3.2. Reflux extraction

The visual appearance of the lavender extract obtained by reflux extraction is shown in Figure 1, highlighting the efficient release of bioactive compounds into the solvent [7].

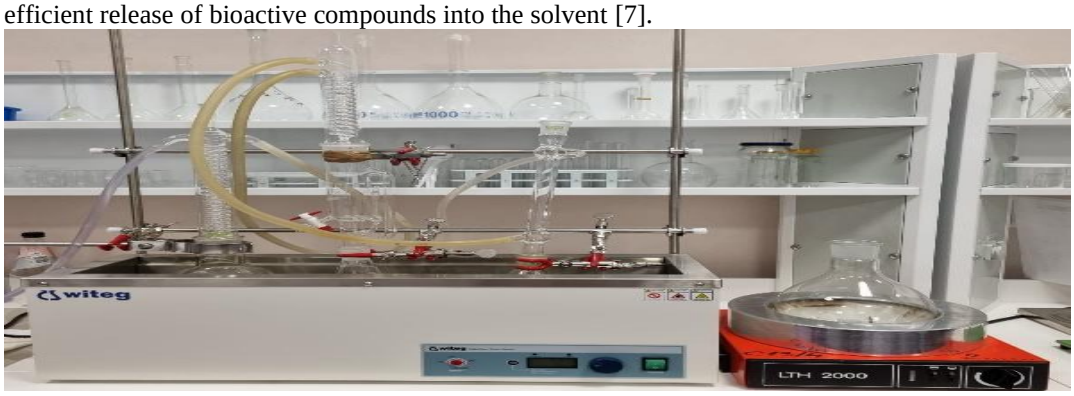

Figure 1. Lavender extract obtained by reflux extraction

3.3. The pH of lavender extract

Figure 2 illustrates the pH values recorded immediately after extraction (a) and after several days of storage (b), indicating a slight acidification associated with the release of phenolic compounds [8].

a)

b)

Figure 2. The pH value: a) after extration; b) after few days

The pH value of the lavender extract gradually decreases from neutral to slightly acid over time, as phenolic compounds are released, indicating a progressive stabilization of the extract.

3.4. Soxhlet extraction

The ethanolic extract obtained using Soxhlet method is presented in Figure 3, showing a more intense colour due to the enhanced extraction efficiency of this technique.

Figure 3. The obtaining ethanolic extract of lavender

3.5. The pH of ethanolic lavender extract

Figure 4 presents the pH variation of the ethanolic lavender extract obtained by Soxhlet extraction, demonstrating the impact of the high-temperature extraction process on extract stability.

Figure 4. The pH of ethanolic lavender extract

4. CONCLUSIONS

Following the experiments performed, significant differences were observed between the extraction methods analyzed in terms of physicochemical parameters. Hot maceration resulted in more concentrated extracts, with slight modifications in density, pH, viscosity and refractive index compared to cold maceration. The density increased slightly due to the higher content of extracted active compounds (by approx. 0.005-0.010 g/cm3), while the refractive index also showed a moderate increase (0.002-0.005 units).

The pH of the aqueous lavender extract gradually decreased from a neutral value to slightly acidic over time, as phenolic compounds were released, indicating progressive extract stabilization. After a few days, the extracts reached a chemical equilibrium following the initial release of active constituents.

Overall, hot maceration can be considered a more efficient method for obtaining extracts rich in active principles, whereas cold maceration is preferred for applications requiring greater stability and subtle sensory properties.

Compared to lavender, rosemary showed a slightly lower yield, which may be related to differences in plant matrix structure and the lower solubility of some terpene compounds in the selected solvent system

In conclusion, the selection of the extraction method should be made according to the intended purpose: preserving sensitive compounds, maximizing yield, therapeutic use or cosmetic formulation. These aspects are essential for the development of effective and safe products based on standardized plant extracts.

REFERENCES

[1] Salmerón-Manzano, E., Garrido-Cardenas, J. A., & Manzano-Agugliaro, F., Worldwide Research Trends on Medicinal Plants. *International journal of environmental research and public health*, *17*(10), 3376, **2020**

[2] Baki, G., *Introduction to cosmetic formulation and technology*. John Wiley & Sons, **2022**

[3] Michalak M., Plant Extracts as Skin Care and Therapeutic Agents. *International journal of molecular sciences*, *24*(20), 15444, **2023**

[4] *Batiha, G. E., Teibo, J. O., Wasef, L., Shaheen, H. M., Akomolafe, A. P., Teibo, T. K. A., Al-Kuraishy, H. M., Al-Garbeeb, A. I., Alexiou, A., & Papadakis, M., A review of the bioactive components and pharmacological properties of Lavandula species. Naunyn-Schmiedeberg's archives of pharmacology, 396(5), 877–900, **2023***

[5] Abubakar, A. R., & Haque, M., Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *Journal of pharmacy & bioallied sciences*, *12*(1), 1–10, **2020**

[6] Romanian Pharmacopoeia, Ed. X, Medical Publishing House, **2021**

[7] Popescu M., Costache G., Mihăilă M.A., Instrumental methods of analysis, Hamangiu Publishing House, **2023**

[8] Mariana POPESCU, Anca Daniela RAICIU. Pharmacognosy and phytochemistry. Practical methods for identification and dosage of active principles from plant materials, Hamangiu Publishing House, **2017**

NAVIGATING FOOD EFFECTS FOR IMMEDIATE-RELEASE PRODUCTS UNDER ICH M13A

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DOI: https://doi.org/10.66793/titum19proceeding23

ABSTRACT

Regulatory expectations for food-effect assessment in immediate-release (IR) products have long diverged: historically, the US FDA broadly expected a fed bioequivalence (BE) study using a high-fat, high-calorie meal, whereas the EMA approach was more variable—often fasting BE unless a meaningful food effect was anticipated, with fed conditions not uniformly specified. Beyond policy, real-world diets differ: in much of Europe and other regions (ex. Japan which is also a ICH adhering country, customary breakfasts are lower in calories and fat than typical US/UK "test meals," complicating global programs and sometimes prompting duplicative studies. ICH M13A provides a pragmatic middle ground. It harmonizes when fed studies are needed for IR products and standardizes the fed condition using explicit caloric/macronutrient targets. M13A also aligns key BE elements (design, endpoints, sampling, analysis), reducing variability due to regional practice. The result is a single, globally acceptable strategy for evaluating food effects that preserves scientific rigor, respects dietary realities, and streamlines development, bridging prior FDA–EMA and other countries differences and enabling efficient worldwide submissions.

INTRODUCTION

For immediate-release (IR) drug products, the impact of food on bioavailability has long been a regulatory flashpoint. Sponsors planning global programs frequently encountered divergent expectations: in the United States, FDA practice consistently centered on conducting a fed bioequivalence (BE) study with a standardized high-fat, high-calorie meal; in Europe, EMA requirements were more context-dependent—often fasting BE by default, with fed assessments triggered when a meaningful food effect was anticipated. The result was an uneven landscape that invited parallel studies, inconsistent meal specifications, and prolonged timelines.

Beyond policy, culture and diet complicated matters. The "test meal" historically used in the US and UK resembles a calorically dense, fat-rich breakfast that is not representative of routine breakfasts across much of continental Europe, Asia, or Latin America. In these regions, customary morning meals are lighter and leaner, raising practical questions about feasibility, tolerability, and clinical relevance. Developers were left to reconcile scientific rigor with regional dietary norms, sometimes at the cost of added complexity or duplicative trials.

ICH M13A changes this equation. As the foundational module in the M13 series on bioequivalence, it offers a harmonized framework for IR products that clarifies **when** a fed study is warranted and **how** the fed condition should be implemented. Instead of prescribing a single, culturally specific menu, M13A defines target caloric and macronutrient characteristics and allows regionally appropriate meals that meet those targets. This "middle-ground" approach preserves the sensitivity of a challenging fed condition while acknowledging legitimate differences in dietary patterns and clinical practice around the world.

Equally important, M13A aligns core BE elements—study design (typically crossover), primary PK endpoints (AUC and C_max), sampling windows, and statistical analysis—so that a single well-designed program can satisfy authorities in the US, EU, and other ICH regions. The guidance reduces ambiguity around meal composition, minimizes avoidable repetition, and provides a common lexicon for regulators, ethics committees, and clinical sites.

This article explains the prior FDA–EMA differences that shaped food-effect strategy for IR products, why regional dietary realities made a one-size-fits-all menu impractical, and how ICH M13A offers a pragmatic solution. We highlight the implications for protocol design, operational execution, and global submissions, and we propose practical tips for implementing M13A's fed condition across diverse settings—without compromising scientific rigor or patient safety.

EMA REQUIREMENTS EXISTENT PRIOR TO ICH M13A

Before ICH M13A, EMA's core reference was the **Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev.1/Corr)**, effective 1 August 2010. For immediate-release (IR) products with systemic action, the **default** expectation was a **single-dose BE study under fasting conditions**, because fasting was considered the most sensitive state to detect formulation differences. If the reference product's SmPC directed intake **only with food**, the BE study was to be run **under fed conditions**. For IR products with specific formulation characteristics (e.g., **microemulsions, solid dispersions**), EMA generally expected **both fasted and fed studies**, unless the product was to be used exclusively in one state. Sponsors could run **two separate two-way crossover studies** or a **single four-way crossover** to cover both states.

When a **fed** study was required but the originator SmPC did **not** specify a meal, EMA recommended a **standard high-fat, high-calorie meal**: **~800–1000 kcal** with **~50% of calories from fat** (approx. **150 kcal protein, 250 kcal carbohydrate, 500–600 kcal fat**). Timing was also standardized: **start the meal 30 minutes prior to dosing and consume it within 30 minutes**. The meal's macronutrient composition had to be documented (grams and kcal).

Operationally, EMA provided additional conduct details: follow the originator SmPC for meal timing if specified; otherwise apply the **30-minute pre-dose start** rule above; standardize posture and activity; and restrict foods/drinks that affect GI/hepatic/renal function (e.g., alcohol, grapefruit). Sampling needed to capture early absorption and ensure **AUC(0–t) ≥ 80% of AUC(0–∞)**; **AUC(0–72 h)** was acceptable for IR forms when appropriate.

Clarifications and case examples were issued via the **PKWP Q&A** document. Notably, EMA reaffirmed that **fasting** is the general rule **even when later data suggest a food effect**, if the originator SmPC permits dosing "with or without food." Conversely, for products like **cyclosporine** (an NTID), EMA required BE **in both fasting and fed states** and applied **narrowed acceptance ranges** in both. These positions underscored EMA's SmPC-anchored approach and risk-based triggers for fed assessments prior to harmonization.

In summary, pre-M13A EMA policy centered on: **fasting by default; fed if the SmPC required it or the formulation warranted it; standardized high-fat/high-calorie meals when fed state was needed; and clear operational rules for meal timing and study conduct.** This framework differed in emphasis from FDA practice and set the stage for the harmonized, globally implementable fed-study specifications later codified in **ICH M13A**.

US-FDA REQUIREMENTS EXISTENT PRIOR TO ICH M13A

Prior to ICH M13A, the FDA's expectations for immediate-release (IR) products were anchored in three guidance pillars: (1) **Food-Effect Bioavailability and Fed Bioequivalence Studies** (2002), (2) **Bioavailability and Bioequivalence—General Considerations** (2003; updated draft 2020), and (3) **BE Studies With PK Endpoints for ANDAs** (most recently revised 2021). Collectively, these documents drove a consistent practice: **evaluate BE under fasting conditions and, for most IR generics, also perform a fed BE study using a standardized high-fat, high-calorie meal**—unless labeling or product-specific guidance clearly indicated otherwise.

Standard fed test meal and timing. FDA specified a challenging fed condition to maximize the likelihood of detecting food effects on rate/extent of absorption. The recommended meal was **high-fat (~50% of total calories) and high-calorie (~800–1000 kcal; often expressed as ~150 kcal protein, ~250 kcal carbohydrate, remainder from fat)**. Subjects began the meal **30 minutes before dosing**, finished it **within 30 minutes**, and the dose was

administered **30 minutes after the meal started** (i.e., immediately upon completion). These specifics were reiterated widely in FDA documents and the clinical literature implementing the guidance.

When to do fed vs fasting. For IR products, the **fasting single-dose, 2×2 crossover study in healthy adults** was the baseline. A **fed BE study** was commonly expected in addition, particularly when product-specific guidance called for it or when food could plausibly alter GI physiology/biopharmaceutics (e.g., low-solubility drugs, bile-dependent dissolution). FDA's ANDA BE guidance framed design/analysis expectations, while product-specific guidances operationalized the need for fed studies.

Design and analysis basics. FDA emphasized **PK endpoints** (AUC, C_max) as the preferred, most sensitive measures for BA/BE when systemic exposure can be characterized, reserving PD or clinical endpoints only when PK is not feasible. BE was assessed using **log-transformed PK, 90% CIs, 80.00–125.00%** acceptance limits. Sampling had to capture early absorption and sufficient terminal data; study conduct (meals/fluids, activity, concomitants) was to be standardized across periods.

Operational details frequently applied pre-M13A.

- **Population:** healthy adults of both sexes, unless safety/ethical concerns dictated otherwise.
- **Meals:** composition documented in grams and calories; standardized menus/site SOPs to ensure compliance with the high-fat specification.
- **Labeling linkage:** Food-effect findings informed **Dosage and Administration** and **Clinical Pharmacology** sections; the 2019 FDA food-effect labeling guidance provided templates to translate exposure changes into clear instructions.

In practice, the FDA's codified **high-fat, high-calorie meal** and routine expectation for **fed BE alongside fasting BE** produced a robust, reproducible framework—but it sometimes clashed with regional dietary norms and EMA's more conditional fed-study triggers. These differences were a key driver for the subsequent **ICH M13A** effort to align *when* to study food effects and *how* to specify the fed condition across regions.

UK REQUIREMENTS BEFORE ICH M13A

Regulatory basis. Prior to ICH M13A, the UK (MHRA) applied the **EMA Guideline on the Investigation of Bioequivalence** (CPMP/EWP/QWP/1401/98 Rev.1/Corr) for immediate-release (IR) products. MHRA documents and FOI responses routinely pointed applicants to this EMA guideline (and its biowaiver appendices) as the UK standard.

Fasted vs fed—when a fed study was required. In line with EMA, the UK default was a **single-dose fasting BE study** for IR products with systemic action. A **fed BE study** was required if (i) the innovator SmPC mandated dosing with food, or (ii) a **clinically relevant food effect** was anticipated (e.g., formulation/biopharmaceutic considerations). Thus, the trigger for fed studies was **conditional**, not automatic.

Fed-meal specification. When a fed study was needed and the SmPC did not define the meal, the UK followed EMA's **high-fat, high-calorie** test meal: **~800–1000 kcal** with **~50% of calories from fat** (≈150 kcal protein, 250 kcal carbohydrate, 500–600 kcal fat), with dosing **30 minutes after the meal starts** and the meal consumed within ~30 minutes. Sites were expected to document macronutrients and timing.

How this differed from the US FDA (pre-M13A).

- **Triggering a fed study:** The **FDA commonly expected both fasting and fed BE** for IR generics, using the same high-fat/high-calorie meal as a sensitive condition; the UK/EMA approach **did not require fed by default**, anchoring the decision to the SmPC and risk of a food effect.
- **Meal composition: Not materially different.** Both jurisdictions used a similar **high-fat (~50%), 800–1000 kcal** meal when a fed study was performed. Differences lay in **policy triggers**, not in breakfast composition.

Why the "UK breakfast" didn't change the rulebook. Although a traditional UK cooked breakfast resembles the caloric/fat profile of the US standard test meal, **UK regulatory practice tracked EMA policy**, not national eating habits. Sponsors ran fed studies **only when justified/required**, but when they did, the **meal matched the EMA high-fat test condition**, effectively mirroring the FDA meal specification.

In the UK, sponsors often submitted **fasting-only BE** unless the SmPC or science mandated a fed arm; in the US, sponsors typically planned **both fasting and fed BE**. This procedural gap—**conditional (UK/EMA) vs routine (FDA) fed studies**—was a recurring source of duplicative designs and regional divergence that **ICH M13A** later set out to harmonize.

JAPAN REQUIREMENTS BEFORE ICH M13A

Regulatory basis. Before ICH M13A, Japan applied the *Guideline for Bioequivalence Studies of Generic Products* issued by MHLW (English translation, 2012) plus an official Q&A (2020). For IR, single-dose, crossover BE in **healthy adults** was the default; fed evaluation was added when labeling or biopharmaceutics indicated a clinically relevant food effect. The core PK framework (AUC, C_max; 90% CI within 80.00–125.00%) paralleled other regions.

When fed studies were run. As in the EU, fed BE was **not automatic** for IR generics; it was triggered by SmPC/Japanese labeling or a plausible food effect based on formulation or BCS/biopharmaceutics considerations. (Japan's guidance also contains unique provisions—for example, enrolling **achlorhydric** subjects when dissolution differences around pH 6.8 suggest sensitivity to gastric acidity.)

Meal composition and timing. When a fed study was required, Japan accepted the standard **high-fat, high-calorie test meal (~800–1000 kcal; ~50% of calories from fat)** and aligned dosing relative to the meal. These specifics appear consistently in PMDA review reports of food-effect/BE studies submitted in Japan.

Operational nuances (timing rules). The 2020 Q&A clarified dosing offsets: products administered **10 minutes after a high-fat meal** (to create a sensitive fed condition); where fasting studies were difficult to conduct, **administration 30 minutes after a low-fat meal** could be used to *minimize* meal effects (primarily discussed in the context of modified-release, but widely referenced operationally).

Convergence with global practice. Comparative surveys before M13 noted Japan among jurisdictions that **define caloric and macronutrient targets** for fed studies—i.e., the same "high-fat/high-calorie" challenge condition used by FDA/EMA—while differing mainly in **when** fed BE was expected (conditional in Japan/EU vs commonly routine in the US for IR).

Summary (pre-M13A).

- **Default:** single-dose, fasting BE in healthy adults for IR generics.
- **Fed BE:** required when labeling or science indicated a meaningful food effect (not by default).
- **Meal:** high-fat/high-calorie (~800–1000 kcal; ~50% fat) with defined dosing offsets; documentation of menu/macros expected.
- **Special cases:** provisions for subjects with altered gastric acidity when dissolution signals warranted.

This landscape—largely aligned with EMA on **triggers** but harmonized with FDA/EMA on **meal specs**—set the stage for ICH M13A to codify a single, globally consistent approach to food-effect and fed BE for immediate-release products.

ICH M13A IN CONTEXT: WHO ICH IS, WHEN M13A TOOK EFFECT, AND WHAT IT CHANGES FOR FOOD-EFFECT STUDIE

The International Council for Harmonisation (ICH) is a global forum that brings **regulators and industry together** to align technical requirements for pharmaceutical development and registration—so a single development program can satisfy multiple authorities. Founding regulators were the **EU (EC/EMA), US (FDA), and Japan (MHLW/PMDA)**; today, the Assembly also includes other regulatory members such as **Health Canada, Swissmedic, MHRA (UK), NMPA (China), ANVISA (Brazil), MFDS (Korea), HSA (Singapore), TFDA (Chinese Taipei), CDSCO (India)** and more, plus industry associations (EFPIA, JPMA, PhRMA, IGBA, etc.).

The guideline and timing

The full title is **"M13A: Bioequivalence for Immediate-Release Solid Oral Dosage Forms."** It reached **Step 4 (adoption by the ICH Assembly) on July 23, 2024**. Regional "in-force" dates vary; in the **EU it came into effect on January 25, 2025**, superseding relevant parts of the EMA BE guideline. FDA has posted its implementation page and finalised adoption of M13A in 2024.

Food-effect (fed/fasted) requirements under ICH M13A

M13A harmonises *when* to run fed vs. fasted BE for immediate-release (IR) products and *how* to standardise the meal:

- **General principle:** Single-dose **fasting BE** usually offers the most discrimination; for **many non-high-risk IR products, one fasting study is sufficient**.
- **Label-driven cases (non-high-risk):**
  - If the comparator is **labelled "only with food" for PK reasons**, do a **single fed BE** study.
  - If it is **"only with food" for tolerability (non-PK) reasons**, a **single study under either fasting *or* fed** conditions is acceptable.
- **High-risk products** (e.g., low-solubility actives in **solid dispersions, lipid-based, nano/micro-emulsion** or other specialised technologies): conduct **both fasting and fed BE** regardless of label, if safety permits.
- **Meal standardisation (fed studies):**
  - Start the meal **30 minutes before dosing** and finish it within **30 minutes**.
  - For studies requiring both states (e.g., high-risk), use a **high-fat/high-calorie meal**: **~900–1000 kcal**, ~**50% fat** (≈**150 kcal protein**, **250 kcal carbohydrate**, **500–600 kcal fat**).
  - For a **single fed study** with non-high-risk products, either the above **high-fat** meal **or** a **low-fat/low-calorie** meal (e.g., **~500 kcal**, ~**25% fat**) may be used—**unless** the comparator's labelling specifies a meal, in which case follow that. Document the meal's macros (grams/kcal/%).

Together, these provisions give a **globally consistent, diet-agnostic** framework—preserving a sensitive fed challenge where needed, while allowing regionally appropriate menus that **meet defined caloric/macronutrient targets**.

CONCLUSIONS

With **ICH M13A** now in force, a food-effect study designed and conducted in line with this guideline can be submitted **across ICH member regions** once implemented locally, eliminating the need to tailor separate protocols to divergent regional expectations. This is a **major advance**: it reduces duplicative development, lowers operational costs, and—most importantly—**spares healthy volunteers and patients** from enrollment in redundant studies that add burden without adding scientific value.

Harmonisation also clarifies an important nuance: **no prior regional approach was "wrong."** Rather, each reflected local practice and dietary habits that were not universally shared. M13A provides a **clear, globally applicable framework**—standardizing when fed studies are needed and how meals should be composed—while still allowing regionally appropriate menus that meet defined caloric and macronutrient targets.

The result is an **ethically stronger and scientifically consistent** pathway for assessing food effects in immediate-release products. Sponsors can now plan a single, well-designed program that **navigates differences in dietary patterns and regulatory expectations** without sacrificing sensitivity or rigor. In short, M13A delivers what global development has long needed: **clarity, efficiency, and ethical prudence** in the design of food-effect studies.

REFERENCES

*1.**European Medicines Agency.*** Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev.1/Corr). *European Medicines Agency*, ***2010***.

*2.**Food and Drug Administration (U.S.).*** Food-Effect Bioavailability and Fed Bioequivalence Studies. *U.S. Food and Drug Administration*, ***2002***.

*3.**Food and Drug Administration (U.S.).*** Bioequivalence Studies With Pharmacokinetic Endpoints for Drugs Submitted Under an ANDA. *U.S. Food and Drug Administration*, ***2021***.

*4.**Food and Drug Administration (U.S.).*** Assessing the Effects of Food on Drugs in INDs and NDAs: Clinical Pharmacology Considerations. *U.S. Food and Drug Administration*, ***2024***.

*5.**International Council for Harmonisation.*** M13A: Bioequivalence for Immediate-Release Solid Oral Dosage Forms. *ICH Secretariat*, ***2024***.

*6.**Ministry of Health, Labour and Welfare (Japan).*** Guideline for Bioequivalence Studies of Generic Products. *Ministry of Health, Labour and Welfare / National Institute of Health Sciences*, ***2012***.

*7.**Ministry of Health, Labour and Welfare (Japan).*** Guideline for Bioequivalence Studies of Generic Products: Q&A. *Ministry of Health, Labour and Welfare / National Institute of Health Sciences*, ***2020***.

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