



# SOCRATIC LECTURES

12 TH INTERNATIONAL SYMPOSIUM, LJUBLJANA, JANUARY 11, 2025  
PEER REVIEWED PROCEEDINGS PART I

EDITED BY:

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## Socratic Lectures

### 12 th International Symposium, Ljubljana, January 11, 2025

Peer Reviewed Proceedings, Part I

Edited by Veronika Kralj-Iglič, Yelena Istileulova and Anna Romolo

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**Program of the Symposium Socratic Lectures, January 11, 2025, 10:00 – 17:00 (Ljubljana time)**

10.00 Welcome to participants (Veronika Kralj-Iglič, University of Ljubljana)

10.05 - 10.45 Plenary lecture: Saara Laitinen: Extracellular vesicles from blood cells and in blood.  
The avenue of future therapies

10.45 Break

**11.00 Scientific sections**

**Section 1: Medicine (Chairs: A. Nemeč Svete, K. Troha)**

11.00 - 11.20 G. Omejec: Ultrasonographic assessment of diaphragm function

11.20 - 11.40 A. Troha: Advancements and future perspectives of platelet-rich products and extracellular vesicles in otology

11.40 - 12.00 A. Čuček: Mammary gland - anatomy, histology, pathology, and physiotherapist management post surgery

12.00 - 12.20 K. Pahovnik: A case of autosomal dominant osteopetrosis with a novel mutation in CLCN7

12.20 - 12.40 L. Dolinar: Destigmatization of erectile dysfunction

12.40 - 13.00 D. Dolinar, I. Potparič: Premature fracture of a modular femoral neck after total hip arthroplasty

**Section 2 : Veterinary Medicine (Chairs: V. Erjavec, M. Šimundič)**

11.00 - 11.20 L. Jarnovič: Brachicephaly in cats. A silent problem in feline health

11.20 - 11.40 B. Perc: Hematology-derived inflammatory markers in dogs with BOAC

11.40 - 12.00 I. Premrl: Foreign body removal from the canine digestive tract: findings and outcomes

12.00 - 12.20 M. Šimundič: Approach to the patient with polyuria/polydipsia

12.20 - 12.40 B. Dučić: Retained tooth roots in cats

12.40 - 13.00 M. Arko: Extracellular vesicles in bovine colostrum and milk

**Section 3: Physiotherapy (Chairs: R. Vauhnik, A. Jakopič)**

11.00 - 11.20 Bec: Effects of transcutaneous electrical nerve stimulation in patients with fibromyalgia syndrome

11.20 - 11.40 K. Breznik: Pain relief treatment of fresh sacral fracture for a professional snowboarder one month before participation in the Winter Olympic Games: a case report

11.40 - 12.00 A. Jakopič: Effects of hydrotherapy on functional capacity of patients after stroke

12.00 - 12.20 M. Jelen: The effects of passive loading of the knee joint after anterior cruciate ligament reconstruction on knee stability

12.20 - 12.40 E. Strle, L. Uhan: Surgical treatment of chronic post-traumatic hamstring origin pain with tendon transfer from the unfused ischial tuberosity apophysis to the ramus of the ischial bone: a case report

12.40 - 13.00 M. Zoroja: Ring shaped lateral meniscus - Raise awareness and avoid unnecessary surgery

**Section 4: Green transition (Chairs: T. Griessler Bulc, M. Bavcon Kralj)**

11.00 - 11.30 A. Drozd-Rzoska: Physics & Society : High Pressures for innovative pro-health foods (keynote lecture)

11.30 - 11.45 A. Kranjc Požar: Microalgal technologies: Treatment strategy for contaminants of emerging concern

11.45 - 12.00 E. Andreasidou: Uptake of contaminants of emerging concern in tomato plants irrigated with treated wastewater

12.00 - 12.15 L. Klemenčič: Harvesting of algae biomass by electrocoagulation

12.15 - 12.30 J. Danilović Luković: Impact of ionizing radiation on microalgae: Enhancing biotechnology potential



- 12.30 - 12.45 U. Šunta: Surface properties of algal biomass and microplastics: An exploration of point of zero charge and contact angle  
 12.45 - 13.00 T. Zrnc Drobñjak: Production of plant biostimulants from microalgae grown on the biogas digestat

**Section 5: Nanostructure pipeline (Chair: P. Trebše, U. Lavrenčič Štangar)**

- 11.00 - 11.10 I. Jerman: Infrared and Raman spectroscopy  
 11.10 - 11.20 J. Jakše: Next generation sequencing  
 11.20 - 11.30 J. Hočevar: NMR Spectroscopy  
 11.30 - 11.40 R. Cerc Korošec: Thermoanalytical techniques  
 11.40 - 11.50 M. Novinec: Mass photometry for molecular mass determination  
 11.50 - 12.00 G. Schlosser: LC-MS lipidomics, metabolomics  
 12.00 - 12.10 E. Heath: LC-MS/MS methods  
 12.10 - 12.20 P. Hansson: SAXS and investigation of diffusion and transport of drug discovery systems in extracellular matrix models  
 12.20 - 12.30 V. Kralj-Iglič: Scanning electron microscopy  
 12.30 - 12.40 O. Vanderpoorten: Nanospacer: Nanofluidic sizing of biomolecules and EVs  
 12.40 - 12.50 G. Liguori: in vitro cell assays  
 12.50 - 13.00 S. Michelini: ELISA assays  
 12.50 - 13.00 All partners: Discussion on sample preparation and characterization

**Section 6: FarmEVs: Different sourced extracellular vesicles and their potential (Chairs: G. Pocsfalvi, A. Di Loria)**

- 11.00 - 11.20 G. Guerriero: Plant extracellular vesicles  
 11.20 - 11.40 I. Schabussova: Bacterial extracellular vesicles  
 11.40 - 12.00 S. Vainio: From sustainable soil to lifelong health - speculations from ecological nanointeractomics  
 12.00 - 12.10 R. Mammadova: Enhancing therapeutic potential: Loading strategies for plant-derived nanovesicles  
 12.10 - 12.20 Glamoclija: Trichinella spiralis extracellular vesicles alleviate OVA-induced allergic inflammation in BALB/c mice  
 12.20 - 12.30 P. Devarkanda: Bean PDNVs isolation and characterization from the dwarf organic Borlotti bean *Lingua di Fuoco*  
 12.30 - 12.40 E. Cepec: Biostimulants from algae biomass uncovered: phytohormones determination and plant growth stimulation  
 12.40 - 12.50 B. Koreñjak: Interferometric light microscopy of diluted blood in different species

**Section 7: Biophysics (Chair: A. Iglič)**

- 11.00 - 11.20 S. Rzoska: Unique properties of high-pressure & high-temperature formed glasses: The application for new-generation batteries (Keynote lecture)  
 11.20 - 11.40 T. Beke Somfai: Peptide-based antibiotic supramolecules (Keynote lecture)  
 11.40 - 12.00 L. Mesarec: Equilibrium membrane shapes influenced by different concentrations of orientationally ordered curved rod-like membrane protein  
 12.00 - 12.20 M. Mussel: On spikes and sound: Debating the physical nature of action potentials  
 12.20 - 12.40 L. Bar: Impact of inclusions on the organization and phase behavior of lipid membrane models  
 12.40 - 13.00 M. Drab: Monte Carlo studies of vesicle shapes with anisotropic membrane inclusions and volume constraints

**Section 8: Physics (Chair: S. Kralj)**

- 11.00 - 11.30 A. Jelen: Microscopic patterns and complexity (Keynote lecture)  
 11.30 - 11.45 A.A. Sojeka: Doomsday criticality for the global society  
 11.45 - 12.00 T. Blazevic: Maximal entropy production  
 12.00 - 12.15 D. Dovnik: Fractional topological charges  
 12.15 - 12.30 J. Sgerm, A. Ribas, L.G. Fugina: Universality of physics of phonons  
 12.30 - 12.45 G. Goričan: Kibble-Zurek mechanism and applications  
 12.45 - 13.00 M. Župec, M. Kodrin, R. Rojko, M. Štorman: Crystal lattices in nature



### Section 9: Liquid Crystals (Chairs: A. Jelen, B. Švajger)

- 11.00 - 11.20 T. Javornik: Importance of the critical point in thermotropic nematic liquid crystals in terms of their sensitivity  
11.20 - 11.40 A. Hoelbl: Localised excitations in liquid crystals as particle analogues  
11.40 - 12.00 E. Čokor: Imry-Ma patterns in confined nematic liquid crystals  
12.00 - 12.20 M. Potrč: Lyotropic liquid crystalline phases  
12.20 - 12.40 B. Švajger: Volume and surface phase transitions in confined nematic liquid crystals  
12.40 - 13.00 M. Zid: Mode coupling and memory effects

### Section 10: Education (Chair: G. Torkar)

- 11.00 - 11.20 G. Torkar: Environmental education for behaviour change  
11.20 - 11.40 I. Devetak: Pre-service primary school teachers' understanding of biogeochemical cycling  
11.40 - 12.00 L. Vinko: Environmental literacy of chemistry teachers  
12.00 - 12.20 S. Beslagic: Robotics in high school  
12.20 - 12.40 T. Plešnik: Undergraduate physiotherapy student perceptions of teaching and learning activities associated with clinical education  
12.40 - 13.00 Ž. Rode: Pre-service teachers as citizen scientists

### Section 11: Bridging Science, Health and Arts I (Chair: Y. Istileulova)

- 11.00 - 11.20 N. Gomes: Transformative educational strategies with artificial intelligence (Keynote lecture)  
11.20 - 11.40 S. De Lasala Porta: Truth, beauty, and ethics in art, science, and health: Interdisciplinary reflections and philosophical perspectives  
11.40 - 12.00 F. Banabed: Exploring health humanities from a global south perspective  
12.00 - 12.20 E. Rosakebia: Balancing care and creation: The role of poetry in caregiving and emotional resilience  
12.20 - 12.40 F. Dalpane: Making art as bravery: A virtue-ethics perspective  
12.40 - 13.00 Y. Istileulova: Planetary health through arts: De rerum natura and Earth's first music in cosmic harmony

### Section 12: Bridging Science, Health and Arts II (Chair: V. Vidrih Perko)

- 11.00 - 11.20 R.S. Thomas: Combining an art therapy-trauma protocol and sensory motor art therapy to support a woman with breast cancer  
11.20 - 11.40 E. Hribernik: Man on the stage of life - introduction to an Italian baroque opera  
11.40 - 12.00 A.L. Mastruzzo: The performer as a multi-expressive artist in Argentina's current flute experimentation  
12.00 - 12.20 A. Karboski: Vladimir Stifitar and his views on the formation of the architectural and cultural landscape of the Eupatoria resort  
12.20 - 12.40 N. Paliska: Mozart iconography  
12.40 - 13.00 V. Vidrih Perko: Learning - the immortal soul of humanism (Keynote lecture)

13.00 Break

### 14.00 Plenary lectures

- 14.00 - 14.30 V. Hlavackova Pospichalova: Extracellular vesicles in ovarian cancer  
14.30 - 15.00 N. Gov: Magnets, Ants and Humans. The physics of collective transport by ants  
15.00 - 15.30 H. Murto: Organic farming  
15.30 - 16.00 E. Mihajlović: Claudio Monteverdi and so called seconda pratica or how he brings real human feelings into music

16.00 Student zone

17.00 Closing of the symposium



**Satellite event: Concert at the Betteto Hall, Academy of Music, Casino Building 10.1.2025 at 18.00**

**Tentative Program:**

**Classical music:**

C. Franck: Petit offertoire. Organ: Yelena Istileulova

C. Monteverdi/G.F. Busenello: Prologue from opera Coronation of Poppaea. Sopranos: Fortuna: Ronja Prapotnik, Virtu: Eva Kokot, Amore: Brina Vukovič, Harpsichord: E. Mihajlovič, Organ positive: Branko Rezič

A. Aljabjev A: Nightingale. Flute: Anita Prelovšek, Piano: Elena Startseva Somun

S. Rachmaninov: Vocalise. Violin: Vasilij Meljnikov, Piano: Lara Oprešnik

C. Monteverdi/G.F. Busenello: Regina disprezzata from opera Coronation of Poppaea. Soprano: Ottavia: Alessandra Tessaro, Harpsichord: E. Mihajlovič, Organ positive: Branko Rezič

S. Rachmaninov: Etude Tableaux Op 33 No 3. Piano: Lara Oprešnik

G. Brun: Romance. Flute: Anita Prelovšek, Piano: Elena Startseva Somun

C. Monteverdi/G.F. Busenello: Duetto Demigella and Valetto from opera Coronation of Poppaea. Sopranos: Demigella: Nives Hadžić, Valetto: Eva Kokot, Harpsichord: E. Mihajlovič, Organ positive: Branko Rezič

W.A. Mozart: Alla turca from Sonate in A major. Piano: Denis Luin

F. Chopin: Polonaise G sharp minor. Piano: Matic Bogataj

G. Ipavec/A. Čopi - Simon Gregorčič: Mountain flower: Chorus Studenec

Poetry by Ifigenia Simonović

Ifigenija Simonović is writing poetry, essays, book reviews, translating, and painting various objects for more than fifty years. She is the author of ten poetry collections, three books of essays, three books for children, and is also known as a publisher of eight books of poetry by Vitomil Zupan. In 2009, she received the Rožanec award. Between 2017 and 2021, she was the president of the Slovenian PEN Center. Her poetry is often dark, but love persistently shines through the cracks that are drawn on her path by real-time experiences.

H. Lavrenčič/Anonymus: The coque has sung: Chorus Studenec

E. Adamič/O. Župančič: Evening song: Chorus Studenec

Chorus Studenec Pivka, led by Irena Rep, is composed of experienced singers. In its 22 seasons, various genres were performed but the singers prefer to sing arrangements of folk songs. They have around 30 concerts annually, home and abroad, most importantly, the visits to Slovenian societies throughout Europe.

**Contemporary music**

A. von Sultanova/Titus Lucrecius: De rerum natura. Piano, Voice: Aleona von Sultanova

S Kralj: Topology. Piano: Samo Kralj

H. Mancini: Pink Panther. Trombone: Emil Somun, Piano: Elena Startseva Somun

A. von Sultanova/A. von Sultanova: For Samuel Gmelin - water, water. Piano, Voice: Aleona von Sultanova

J. Rae: Sonatine (Aquarelle, Notturme, Firedance). Flute: Anita Prelovšek, Piano: Elena Startseva Somun

A. Schnittke: Suite old style. Violin: Branko Brezavšček, Piano: Elena Startseva Somun

L. Oprešnik: Fugue. Piano: Lara Oprešnik

B. Kobal: Dic verbo. Soprano: Kaya Tokuhisa, Organ: Jana Jamšek

D. Zupanič Turkovič: Cantique de Baruch Spinoza from Mass in E minor. Organ: Aleona von Sultanova, Percussion: Bojan Ilievski, Piano: Lara Oprešnik, Flute: Anita Prelovšek, Voice: Veronika Kralj-Iglič

**Satellite event: Recital of the organist Roberta Schmid at the Church of Assumption, Tromostovje 14.1.2025 at 20.00.**

**Program:**

D. Buxtehude: Passacaglia in D minor BuxWV 161

J. S. Bach: Choral Das alte Jahr vergangen ist BWV 614

J. S. Bach: Choral Erbarme dich mein Gott BWV 721

A.G. Ritter: Sonate no. 2 in E minor op.19

G. Mushel: Toccata

J. Rheinberger: Passacaglia

L. Vierne: Carillon de Westminster



## Editorial

12th Socratic Lectures symposium took place online on January 11, 2025. It featured four plenary lectures, 12 scientific sessions, a poster session, a newly introduced session called "The student zone". The estimated number of the participants was around 300. The symposium opened with an inspiring lecture "Extracellular vesicles from blood cells and in blood. The avenue of future therapies" by Saara Laitinen. The afternoon session was composed of four plenary lectures: "Extracellular vesicles in ovarian cancer" by Vendula Hlavackova Pospichalova, "Magnets, Ants and Humans. The physics of collective transport by ants", by Nir Gov, "Organic farming" by Henry Murto and "Claudio Monteverdi and so called *seconda pratica* or how he brings real human feelings into music" by Egon Mihajlović.

Saara Laitinen took a master degree in Plant physiology and a PhD in Biochemistry. She followed her academic career at the University of Helsinki, then at the Institute Pasteur de Lille, France, and at distinguished research institutes in Helsinki. Presently she is a research and development manager at the Finnish Red Cross Blood Service. She is responsible for the laboratory facilities, research, and development within scientific projects. She has been working on extracellular vesicles for many years and is integrated into the International Society for Extracellular Vesicles (ISEV) founded in 2012. Her initial interests were extracellular vesicles from mesenchymal stromal cells, but now she focuses on the immune regulation of blood cells. Saara Laitinen was the president of the Fin Society for Extracellular vesicles and is presently its vice president.

Nir Gov is a physicist, working at Weizmann Institute in Rehovot, Israel. He is developing theoretical models of biological phenomena and related systems. These models consider the laws of statistical physics, non-linear dynamics, and classical theories. Since living systems seem complex, it is the strength of the models to find the most relevant parameters by which the system's behavior can be predicted and prof. Gov was able to point to the features that show general validity in systems that at first sight seem very different, as for example magnets, ants, and humans.

Vendula Hlavackova Pospichalova took her bachelor degree, master's degree, and PhD at the Charles University in Prague. She was a postdoctoral fellow at the Institute of Molecular Genetics, Prague and a one year visiting researcher at the Duke University, North Carolina, USA. Presently she is a teacher and a researcher at the Masaryk University in Brno. She is a member of the International Society of Extracellular Vesicles and the president of the Czech extracellular vesicles society. Her interests are the role and mechanisms of extracellular vesicles in cancer.

Henri Murto has a professional farming background of 20 years and he is specialized in organic and biodynamic (regenerative) farming methods. Henri runs the Lehtosarvi farm together with his wife. The main focus of the farm is training, research, and growing medicinal plants. He is active lecturer and writer and is a member of the team of the FarmEVs, a project funded by European Research Executive Agency (REA) within Marie Skłodowska-Curie Actions & Support to Experts, Staff Exchanges action No. 101131175. The leading institution is the National Research Council of Italy, Institute of Biosciences and Bioresources, Napoli, and the principal investigator of the project is the Head of the Extracellular Vesicles and Mass Spectrometry Group dr. Gabriella Pocsfalvi. The Lehtosarvi farm is one of the 8 partner institutions from 6 countries. Based on the research of extracellular vesicles, FarmEVs strives to introduce organic plant-derived extracellular vesicles into sustainable agriculture with the aim of a greener, healthier future for all living things. The symposium featured also latest developments of the FarmEVs project, presented in a special session.

Egon Mihajlović studied harpsichord and early keyboard instruments at the Conservatory of Music and Performing Art in Frankfurt where he also concluded his postgraduate specialization. Since 1990, he has performed as a harpsichordist, a conductor, and an artistic director for baroque opera and sacred baroque music at major concert venues and festivals across Europe and the United States. He was teaching at musical arts institutes of higher education and musical academies in Wurzburg, Nurnberg, Cetinje, and Pesaro. Presently he is a professor for harpsichord and the Head of the Department for Early Music and the head of the department at the Academy of Music of the University of Ljubljana. In 2013, he was awarded the Gold Plaque of the University of Ljubljana for exceptional contributions in the development of scientific, teaching, or artistic endeavors.



We are especially happy to see the students attending the symposium; this time a large group of students from Egypt was included thanks to Maneea Moubrak, the member of the FarmEVs project team. The students of physiotherapy from the Faculty of Health Sciences have prepared a review on mammary gland and breast cancer physiotherapy mentored by Raja Dahmane Gošnak which is being published in this issue. Three more papers were prepared by the students from the Faculty of Health Sciences and from “Fizioterapevtika”.

Extracellular vesicles have been the red line of Socratic lectures since the first one in 2008 by Bernd Engelmann and also this year, the contributions from this field were numerous and excellent. Gabriella Pocsfalvi chaired a session devoted to the Marie Curie Sklodowska mobility project FarmEVs on plant extracellular vesicles. Besides life and natural sciences, we were fortunate to have contributions from social sciences, economy, education, and arts. The students and professors of the Academy of Music presented parts from the opera Coronation of Poppaea by Claudio Monteverdi, which will be put on stage in the summer of 2025. Its production is linked to the project Nanostruktururome including 9 faculties of the University of Ljubljana and its Academy of Music as well as 9 external partners from home and abroad.

As we received more than 40 papers, The Proceedings of the 12th Socratic Lectures is organized in three parts. Part I contains mainly papers from the medical fields, Part II contains mainly papers from the natural and social sciences and Part III contains standard operating procedures of a pipeline for assessment of extracellular particles. The pipeline was designed within the project Nanostruktururome. Socratic lectures thus present an important meeting point for the participants of various projects and for the publication of scientific results, opinions, repositories, and any kind of documents that are useful in the management of the projects. But in the first place, the 12th Socratic lectures are a meeting point for scientists, artists and friends from many different fields. It the recent events again we were able to enjoy the fruit of the fields which were kindly provided by the participants for which we remain forever thankful.

As Socratic lectures are a part of the Z-STEAM (Science, Technology, Engineering, Art, Mathematics) activities, also art is an important element. There were three satellite events starting on January 8 with a competition-awarded performance of the team member Lara Oprešnik at the Musikverein in Vienna. She presented the first part of the Skrjabin concerto in F sharp minor for piano and orchestra at the Brahms hall, accompanied by the Vienna City Orchestra directed by Christian Schulz - within the New Year concert events. Two days later, on January 10, she joined the artists at the Betteto hall in Ljubljana where the networking event of the Socratic lectures symposium took place. The event included classical and contemporary compositions, with an intermezzo of poetry by the renowned Slovene poet Ifigenija Simonović. The recitation was intertwined with Slovene chorals performed by the sborus Studenec. The 12th Socratic lectures ended with an organ recital by Roberta Schmid from Naples, Italy, at the Church of Assumption, Tromostovje, on January 14. Proceedings of the Socratic Lectures hosts a visual art gallery Marguerite de Saint Champs. This time, the presenting artist is Roberta Ferranti from Trieste, Italy. Primarily focusing on street photography, landscapes, geometries, and portraits, Roberta Ferranti seeks to share the beauty and stories that often go unnoticed. Her images challenge viewers to pause and reflect, to see the world from a different perspective, and to appreciate the subtle moments that make life extraordinary. We are thankful to Drago Videmšek for recording the concert at the Betteto hall on artistic photographs and to Miha Červ who attended the concert at the Musikverein, Vienna to report on the concert.

With the participation of world top scientists from the fields and integration of science in international artistic production, Socratic lectures strive to present scientific and artistic excellence to the students and involve them in the creation of science and art. We conclude with a big thanks to all the participants who in the spirit of Socrates donated their contributions and to all those who made the events possible. Kindly invited to the next Socratic lectures.

Veronika Kralj-Iglič, Anna Romolo and Yelena Istileulova



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Review

# Advancements of Platelet-Rich Products and Extracellular Vesicle Use in Otology

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## Abstract:

Autologous blood-derived platelet-rich products exert their regenerative effects by modulating molecular processes involved in hemostasis, inflammation, angiogenesis, cellular transport, cytoskeleton synthesis and other. Abnormalities in these biological processes can be observed in many conditions, including otologic diseases. The action of bioactive components of extracellular vesicles and platelets in platelet-rich preparations presents an additional therapeutic possibility in managing several otologic pathologies, especially where other interventions for enhancing wound healing, tissue regeneration, and reducing inflammation have been exhausted. The application of platelet-rich preparations has been explored in chronic middle ear disease, tympanic membrane perforation, sudden hearing loss, facial nerve damage and other. Furthermore, extracellular vesicles, which are present in all bodily fluids including platelet-rich preparations, have been increasingly investigated as a potential diagnostic and target therapeutic tool in the management of hearing loss.

**Keywords:** Platelet-rich Plasma; Extracellular vesicles; Otology; Growth Factors; Hearing Loss

## 1. Introduction

By providing small particles to tissues and aiding in establishing an optimal molecular microenvironment for tissue regeneration, platelet-rich products have been utilized across various fields of medicine yielding favourable results (Troha et al., 2023). Otolaryngology, a subspecialty focused on the diagnosis and treatment of ear-related disorders, is no exception in the attempts of exploring the beneficial effects of these products.

Platelet rich products are most commonly described in the literature as platelet-rich plasma (PRP) or platelet and extracellular vesicle-rich plasma (PVRP), the latter emphasizing the importance of extracellular vesicles (EVs) in these fluids. PRP and PVRP are autologous blood-derived products with platelet concentrations 3-5 times higher than whole blood (Marx et al., 2001; Tao et al., 2017). EVs are a heterogeneous group of cell-derived membranous structures, harvested from any bodily fluid, including PRP and PVRP. Depending on their origin, EVs play a role in physiological and pathological processes and can be categorized into endosome-origin exosomes and plasma-derived ectosomes (further divided into microvesicles, microparticles, ectosomes, large oncosomes and apoptotic bodies). These nano- and microvesicles are especially recognized for their involvement in membrane and cytosol transport, as well as RNA delivery, the mechanisms by which EVs are being investigated for their potential use in the diagnosis and treatment of a range of diseases (Tao et al., 2017; Dai et al., 2020; Raposo et al., 2013; Steiner & Battelino, 2020; Sluga et al., 2021). In addition to EVs, platelets have been identified as the central mediators of beneficial effects of platelet-rich preparations, participating in essential processes of hemostasis, inflammation, angiogenesis, tissue anabolism, extracellular matrix synthesis, as well as in immune system modulation (Morrell et al., 2014; Anitua et al., 2004). The main bioactive molecules promoting healing in platelet-rich preparations include growth factors, cytokines and other compounds (Anitua et al., 2004; Sundman et al., 2011). The preparation of platelet-rich products is cost-beneficial, straight-forward and involves simple centrifugation, performed either in one or two steps. After centrifugation, platelets in the preparation can be activated either exogenously (using chemical substances like calcium chloride) or endogenously (in vivo after application). A significant advantage of exogenous application is the formation of a gel-like consistency, which facilitates application onto tissues. However, its application should occur within 10 min after activation, in the time frame where most of the beneficial constituents are released (Marx et al., 2001; Croisé et al., 2020; Božič et al., 2021). Platelet-rich products have been found as inherently safe to use in tissues, acting similarly to a blood clot (Andia et al., 2013). Studies investigating possible oncogenic effects (Marx et al., 2001; Marx et al., 1998), or long-term use in animal models (Omar et al., 2017), as well as in sensitive tissues, such as in the middle ear (Arslan et al., 2022) have shown no adverse effects. Instead, the investigations demonstrated significant anti-inflammatory and regenerative potential (Arslan et al., 2022). In addition, it has been shown that the preparations can be tailored in their composition to optimize its therapeutic effect depending on the clinical context, especially regarding the presence or absence of the inflammation process, and on the patients' own blood characteristics (Sundman et al., 2011; Steiner et al., 2022).

## 2. Application of PRP/ PVRP in otology

Studies investigating the tissue regeneration and anti-inflammation functions have made platelet-rich preparations a promising adjunctive treatment modality in otologic diseases, such as middle ear diseases (e.g., tympanic membrane perforation, chronic otitis media), nerve preservation (sudden sensorineural hearing loss, chorda tympani nerve manipulation, facial nerve injuries) and other (Sherif et al., 2024; Mandour et al., 2019; Lee et al., 2017). Some examples and findings of clinical studies are presented in the **Table 1**.



**Table 1.** Examples of studies with PRP/PVRP application in otology. PRP; platelet-rich plasma, PVRP; platelet and vesicle-rich plasma.

Authors and year of publication, type of study and number of subjects	Use in otology	Results
Shiomi and Shiomi (2020): retrospective study, 118 patients; Fouad et al. (2018) : retrospective study conducted, 69 patients; El-Anwar et al. (2015) randomized controlled trial, 64 patients; Mandour et al. (2019) : prospective randomized controlled study, 50 patients; Huang et al. (2021), meta-analysis, 8 studies; Al-Arman et al. (2024) : randomized clinical trial, 156 patients.	Tympanic membrane perforation – use in myringoplasty	<ul style="list-style-type: none"> <li>Significantly improves healing of perforations, also suitable for larger perforations.</li> </ul>
Kanaujia et al. (2023): prospective interventional study, 70 patients; Dave et al. (2021) : case series, 40 patients; Tom et al. (2022): prospective observational study, 54 patients; Tyagi & Rout (2019): Case series study, 200 patients; Arslan et al. (2022) : experimental animal study.	Sudden sensoryneural hearing loss	<ul style="list-style-type: none"> <li>Substantially improves hearing in acute hearing loss.</li> <li>One-time intratympanic injection yields better results compared to intratympanic steroids, no complications.</li> <li>Favourable results in younger patients.</li> <li>Proven safe use in the middle ear.</li> </ul>
Askar et al. (2021): case series, 21 patients. Jang et al. (2016): experimental animal study.	Mastoid reconstruction after CWD (canal wall-down) mastoidectomy	<ul style="list-style-type: none"> <li>Good healing and epithelization of radical cavity.</li> <li>Better visualisation upon follow-up imaging as in obliteration with other materials.</li> </ul>
Vozel et al. (2021): randomized controlled trial, 22 patients.	Chronic discharging radical cavity	<ul style="list-style-type: none"> <li>Successful outcomes in cases of standard therapy failure.</li> </ul>
Lee et al. (2017): case study, 1 patient.	Pinna replantation site	<ul style="list-style-type: none"> <li>Supports neovascularization and transplant protection after reperfusion ischemia.</li> </ul>

### 2.1. Tympanic membrane perforation

Acute and chronic tympanic membrane perforations in acute or chronic form, traumatic or as a consequence of chronic otitis media present a common otologic disease often requiring a surgical intervention. Tympanoplasty is a surgical procedure with reported success rates from 75% to 98% (Mohamad et al., 2012; Sheehy et al., 1980), but tends to be less effective in patients with larger perforations, in those with early onset of middle ear discharge postoperatively, and in patients with myringosclerosis. Especially in cases of recurrent perforations, additional support (such as cartilage graft) is usually provided to the most commonly used temporalis fascia graft. As a means of enhanced aid to graft incorporation and therefore in promoting the closure of the perforation several studies have demonstrated the beneficial effects of PRP and PVRP (Singh & Jain, 2024). Huang et al. (2021) performed a systematic review and meta-analysis, where the efficacy of PRP in myringoplasty was investigated. The analysis included eight studies involving 455 patients. The results showed a significant increase in the closure rate for PRP-treated patients compared to conventional surgery, indicating that PRP significantly enhances the healing process. Moreover, the use of PRP was associated with a lower incidence of complications. However, no significant differences in hearing in patients with closed tympanic membrane improvements between PRP and conventional methods were noted (Yadav et al., 2018). Yadav et al. (2018) demonstrated the use of PRP as applied in an underlay technique between temporalis fascia and tympanic membrane in a study of 20 patients, reporting a significant difference in graft uptake in favour of the PRP group compared to the control after 3 months (Yadav et al., 2018). Akash et al. (2023) conducted a RCT on 40 patients with the use of PRP as packing material in type 1 tympanoplasty in chronic otitis media and 40 in the control group, observing the status of graft uptake and re-perforations at 1st and 6th month postoperatively. The results showed similar graft uptake and re-perforations as well as the rate of post-operative infections in both groups (Akash et al. 2023). In a study by Yousaku Shiomi & Yoshiko Shiomi (2020), the researchers assessed the outcomes of myringoplasty using PRP and atelocollagen sponge foams and found that PRP significantly improved the rates of closure, especially for small perforations. The cause and duration of perforation were not predictors of the outcome, while the patient age was significantly correlated to the surgical success. Subjects older than 80 years had a significantly lower success rate than younger patients (Yousaku Shiomi & Yoshiko Shiomi, 2020). Fouad et al. (2018) compared the outcomes of fat graft myringoplasty (FGM) with PRP or with hyaluronic acid (HA) versus to FGM alone in patients with medium-sized perforations. They reported a significantly higher success rate (85.7%) in patients with PRP than those with FGM alone (60%) (Fouad et al., 2018). El-Anwar et al. (2015) supported the favourable results of PRP use by reporting a 100% success rate in myringoplasty with conchal perichondrial graft with PRP in 32 patients, compared to 32 patients without PRP, all with large dry central perforations. 81.25% success rate was reported in the control group. Additionally, in the test group, a reduced incidence of postoperative infections was noted (El-Anwar et al., 2015). Mandour et al. (2019) compared PRP-enriched fat grafts and reconstruction with cartilage perichondrium in 50 patients undergoing myringoplasty and demonstrated that both approaches yielded comparable results, with an 88% closure rate in the PRP-fat graft group and 92% in the cartilage group. The researchers concluded that PRP addition in fat-graft myringoplasty can be recommended as first line treatment of medium-size central perforations of the tympanic membrane. They additionally proposed that the preparations are effective in transplant durability by preventing dehydration at the margins of tympanic membrane perforations (Mandour et al., 2019). Choudhury et al. (2024) investigated the use of fat grafts combined with PRP in small and moderate perforations in 36 patients and reported a high success rate, suggesting that the convention of using the temporalis fascia graft for smaller perforations should be revisited (Choudhury et al. 2024). Similarly, Al-Arman et al. (2024) compared the efficacy of platelet-rich fibrin-augmented fascia to established cartilage tympanoplasty for large perforations in a RCT study of 156 patients. Graft take and hearing outcomes showed no significant difference, with no complications reported, suggesting PRP as a comparable option without the need of cartilage harvest. Braccini et al. (2009) previously reported a success rate of 96% in tympanoplasty using leucocyte- and platelet-rich fibrin (L-PRF), compared to 85% successful outcomes in tympanoplasty without PRF. The researchers proposed that PRF provides both mechanical

and inflammatory protection to tympanic grafts (Braccini et al., 2009). Sankaranarayanan et al. (2013) found that the PVRP clot application during tympanoplasty prevented graft displacement, facilitating the closure of the perforation.

### 2.2. Sudden sensorineural hearing loss and other nerve impairment in otology

Sudden sensorineural hearing loss (SSHL) is an abrupt deterioration of hearing for at least 30 dB in 3 or more consequent frequencies upon audiometric evaluation, in the time period of 72 h. It is termed idiopathic when the etiology remains unclear after clinical (otologic and audiologic) examination and patient history. Researchers Ding et al. (2009) priorly suggested that the application of PRP has potential regenerative effect on cranial and peripheral nerves, which was later confirmed in different animal and clinical studies (Shen et al, 2019; Li et al., 2019; Kuffler et al., 2011). Sanchez et al. (2017) proposed that platelet-rich products influence nerve repair by mechanical protection, neuron apoptosis prevention, stimulation of angiogenesis, axon regeneration promotion and inflammation modulation in the microenvironment of nerve cells. Singh & Jain (2020) reviewed the role of platelet-rich product use in management of sensorineural hearing loss and concluded that may facilitate the repair and regeneration of damaged auditory cells and nerve fibers, potentially improving hearing function in individuals with SNHL. Arslan et al. (2022) also investigated in an animal model the effects of PRP application intratympanically and found no adverse effects, with lower degrees of inflammation and mucosal thickness versus the control side, and with more evident angiogenesis on the tested side. They suggested that PRP is a safe alternative to current treatment with its the anti-inflammatory and regenerative features. Mahmoud Shawky (2024) conducted a study, where intratympanic PRP injections were compared to steroid intratympanic injections in managing SSHL. A significant improvement in hearing after 2 weeks for 25 dB and after 2 months for 30 dB was observed in the PRP group. Tyagi & Rout. (2019) similarly previously reported patients with mild to moderate SNHL to recover significantly after intratympanic PRP injection, especially in younger patients. Stephy Maria Tom et al. (2022) reported 54 cases with sensory neural hearing loss treated with PRP or dexamethasone intratympanically. In the PRP group, improvement from baseline was significantly higher in PRP group compared to dexamethasone group. Kanaujia et al. (2023) administered intratympanic PRP injections in 70 patients, where hearing loss was present for less than 6 months, of which 85.2 % recovered completely and 14.8% partially. Intratympanic PRP injection significantly improved hearing in acute mild-to-severe cases of sensory hearing loss with no complications reported. Sherif et al. (2024) conducted a comparative study of intratympanic steroid and PRP injection and found no significant differences in treatment modalities, both proving to be similarly effective in improving hearing outcomes in sudden sensorineural hearing loss. Additionally, PRP's neurotropic effects were investigated in cases of facial nerve dysfunction, whether after salivary gland or temporal bone surgery (Bitenc Zore et al., 2022). Ricci et al. (2019) and Scala et al. (2014) have shown that the application of PRP in gel form after superficial parotidectomy reduces the occurrence of postoperative facial palsy. In the study of Cho Hyong-Ho et al. (2010) PRP in combination with neural-induced mesenchymal stem cells was administered to the facial nerve in animal models, showing significant facial nerve regeneration. These effects were attributed to neurotrophic growth factors such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). Moreover, with the idea to aid nerve regeneration and reduce postoperative sensory impairment, an unpublished study is currently underway evaluating the use of PVRP on the chorda tympani nerve, responsible for taste sensation in the anterior two-thirds of the tongue and is commonly stretched or damaged during middle ear surgeries, resulting in taste and other sensory disturbance in the tongue.

### 2.3. Radical cavity reconstruction and chronic radical cavity inflammation

In cases of chronic otitis media with cholesteatoma and subsequent need for surgery, often a radical cavity is formed, which is an operative hollowing of temporal bone performed in canal wall down mastoidectomy surgery. This postoperative cavity can be afflicted with complications such as chronic inflammation with discharge. A study by Elbary et al. (2018) demonstrated the benefits of combining titanium mesh and PRP with bone material in the reconstruction of the posterior meatal wall in middle ear cholesteatoma surgery. This

method was found to be reliable, without complications, giving a smooth appearance to the area and improving healing. In order to aid the epithelization of the radical cavity and reduce the inflammation, Askar et al. (2021) used PRP in addition to bone pate in mastoid reconstruction and demonstrated improved healing, reduction of incidence of complications and better visualization during follow-up imaging for recurrences. Similarly, Vozel et al. (2021) in randomized controlled trial described PVRP as an effective treatment modality for chronic postoperative temporal bone cavity inflammation. For obliterating surgical cavities, Zwierz et al. (2024) used injectable platelet-rich fibrin on a temporoparietal fascial flap in three cochlear implant patients after subtotal petrosectomy due to chronic discharging ears and concluded that this material may reduce the risk of potential infection in the obliterated cavity, in patients without cholesteatoma.

#### 2.4. Ototoxicity

The protective effects of PRP against ototoxicity have also been documented. It was reported that intratympanic administration of PRP can mitigate the ototoxic effects of cisplatin in experimental models, alluding to the potential to preserve auditory function (Yurtsever et al., 2020). In fact, growth factors present in PRP, such as Insulin-like Growth Factor-1 (IGF-1) were suggested to play a role in protecting hair cells from ototoxic damage (Dave et al., 2021).

#### 2.5. Other uses

Lee et al. (2017) described a case of a complete ear amputation in a patient, where PRP was injected along with hyperbaric oxygen therapy and polydeoxyribonucleotide, demonstrating facilitation of neovascularization and protection against transplant failure due to reperfusion ischemia with almost complete salvage of the pinna.

### 3. Advancements in treating sensorineural hearing loss: EVs and autologous platelet-rich preparations

Sensorineural hearing loss is a result of multiple causes, including genetic predisposition, infections, ototoxic agents, exposure to noise, aging. It presents a substantial clinical challenge with no effective pharmacological treatments, despite the high prevalence of the condition globally. As to act to prevent the loss of hair cells or auditory neurons, modern research focuses to protect and regenerate cochlear cells before cell loss. In efforts to establish restoration of cochlear synapses, neurotrophins such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) were proposed (Fritzsch et al., 2004). These regulate the connection between hair cells and auditory neurons during embryogenesis and stabilize the cochlear synapses (Bailey et al., 2014; Warnecke et al., 2020). It has been demonstrated that especially a various composition of neurotrophins has the ability to increase the survival of auditory neurons, compared to single factors alone. Treatment with EVs have been proposed as therapeutic agents, especially containing the mentioned neurotrophins, BDNF and NT-3. PRP and autologous mononuclear cells derived from human bone marrow have been investigated as sources of these factors, since a balanced combination of various factors are naturally occurring in these preparations (Schwieger et al., 2015; Kranz et al., 2014; Kaiser et al., 2013). In addition to a potential therapeutic role, Wong et al. (2018) reported that EVs from rats' primary cultured inner ear cells release exosomes, which could be used as a biomarker showing the state of the inner ear, as the change in EVs' cargo and concentration was noted by exposure to cisplatin or gentamycin. The authors also suggested the isolated EVs could be loaded with anti-inflammatory drugs, therefore, the vesicles could be used as nanocarriers. Zhuang et al. (2021) demonstrated EVs in human perilymph, which were carrying hair cell-specific proteins and suggested that sensory hair cells themselves were the potential source of exosomes. The isolated EVs had specific miRNA and protein cargo profile, derived from different developmental stages of the inner ear postnatally. The study also postulated that these EVs may reflect the physiological and pathophysiological processes in the inner ear (Jiang et al., 2022). This was demonstrated further on examples of EVs from vestibular schwannoma (VS) cell culture. Exosomes from VS cell cultures were shown to likely damage hair cells and auditory neurons from patients with VS and poor hearing, than those of EVs from patients with VS and good hearing (Soares et al., 2016). Protective



functions of EVs have also been investigated such as in protection of hair cells against aminoglycoside toxicity, where researchers used utricle-derived exosomes (Breglio et al., 2020). Moreover, Warnecke and colleagues in 2020 showed that mesenchymal stem cell (MSC) EVs contain neurotrophic factors and that the application of these EVs to the cochlea protected auditory hair cells from noise-induced trauma in vivo. The same researchers first introduced in-human intracochlear application of allogeneic human stromal derived extracellular vesicles during cochlear implantation (Warnecke et al., 2020). Tsai et al. (2021) subsequently showed that the administration of umbilical cord-MSC-EVs to cochlear hair cells in mice after repetitive cisplatin injection improved hearing. In addition, EVs have been investigated in neuro-otology as compounds of viral vector gene therapy, which was previously inefficient in the delivery of genes to all the hair cells of the inner ear. By a vector called exosome-associated adeno-associated-virus (exo-AAV), it was demonstrated that the genes were significantly delivered to all inner ear hair cells, inner and outer. It was shown that AAV with exosomes rescued hearing in a mouse with hereditary deafness (György et al., 2017).

### 3.1. Adverse effects of use in middle ear

As noted previously, the adverse effects of platelet-rich products are extremely rare, there are no concerns about transmittable disease, since the preparation is autologous. Studies have shown that the slightly acidic pH of PRP compared to a matured blood clot, PRP acts inhibitory to bacterial growth, which is beneficial in PRP applications. To assess the safety of the use in the middle ear Arslan et al. (2022) demonstrated a safe prolonged use on the middle ear mucosa, with degrees of inflammation and mucosal thickness significantly lower in middle ear treated with PRP compared to the control side treated with saline. In fact, favourably, the degree of angiogenesis was significantly higher in the PRP administered side, promoting regeneration.

## 4. Conclusions

Autologous platelet-rich blood-derived products are versatile and effective treatment options in otologic disease management with minimal adverse effects due to the autologous nature. The components of the preparations, released in a manner similar to blood clot formation, are involved in healing promotion, inflammation reduction, cell regeneration and angiogenesis. These effects are being used in treatment of sudden sensorineural hearing loss, tympanic membrane perforation, ototoxicity and others, making it a valuable adjunct in clinical otology practice. In recent time, it has additionally been shown that the natural composition of small particles called extracellular vesicles can be used as vectors for gene therapy in transport to all inner ear hair cells, which previously failed, potentially treating sensorineural hearing loss. In the future, personalized protocols with preparations adapted to the clinical case may be expected. Further combinations of platelet-rich products with other adjuvants, such as PRP with gene therapy, stem cells, biomaterials, may be developed in order to enhance the beneficial clinical outcomes. Further studies are needed to support the existing hypotheses.

**Conflicts of Interest:** The authors declare no conflict of interest.

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*Reflection*

# Reflections on Plenary Lecture “Extracellular Vesicles from Blood Cells and in Blood; The Avenue of Future Therapies” by Saara Laitinen at 12<sup>th</sup> Socratic Lectures

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**Abstract:**

Extracellular vesicles have been the scope of the Socratic Lectures since 2008 keeping the interested parties and the students informed on recent developments in the field. The first plenary lecture on extracellular vesicles at the recent symposium was by a distinguished researcher Saara Laitinen. In this contribution, the lecturer is shortly presented, contents of the lecture as reflected by the students are given along with images of extracellular vesicles from blood, and references selected by the lecturer.

**Keywords:** Extracellular vesicles; Extracellular particles; Microparticles; Microvesicles; Blood extracellular particles; Plasma extracellular particles

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## 1. Introduction

The plenary lecture Extracellular vesicles from blood cells and in blood; The avenue of future therapies was presented at the symposium 12<sup>th</sup> Socratic Lectures that took place January 11, 2025 online. It was organized by the Centre of Lifelong Learning of the Faculty of Health Sciences, University of Ljubljana as a part of Z-STEAM activities (Science, Technology, Engineering, Arts and Mathematics). Z-STEAM activities are centred on the students and Socratic Lectures are a part of the curriculum that features top scientists from different fields for students of all levels. Also, Socratic Lectures are constitutive parts of the projects FarmEVs, Nanostructurome, J3-60063, and the program P3-0388. We are indebted to excellent scientists for including students into the discourse and thus enable them to take a part in one of the research segments. The students have attended the symposium and recorded the lectures. They have combined their impressions and the informations from the recording. Images of blood cells and extracellular vesicles (EVs) (Figures 1 and 2) are added for illustration.

## 2. Saara Laitinen: the presenter of the plenary lecture Extracellular vesicles from blood cells and in blood; The avenue of future therapies

Saara Laitinen took her Master of Science degree from Plant Physiology and her PhD at the University of Helsinki, Department of Biosciences, Finland. Her career started at the University of Helsinki and developed at distinguished institutions L'Institut Pasteur de Lille, France, Research Institute Minerva, Biomedicum, Helsinki and Department of Molecular Medicine, National Public Health Institute, Helsinki. Currently, she is a Research and Development manager at Finland Red Cross Blood Services. She is a principal investigator and responsible person for strategic research topic: Blood Cells and Products. She is responsible for the laboratory facilities at the Research and Development Department providing and maintaining the high-quality platform for all four strategic research focus projects currently running in the Finnish Red Cross Blood Service.

Laitinen has over fifteen years of research experience on EVs and is an internationally recognised blood EV researcher participating in the work of the International Organisation Committee for Blood EV Workshop of ISEV (International Society of Extracellular Vesicles). Her interest in EVs started from the mesenchymal stromal cells (MSC). Her current research focus is on the immune regulation of blood cells, platelets, and red blood cells and the role of the membrane lipids in the functionality of the cells and the extracellular vesicles.

Laitinen was a principal investigator of numerous projects, publications and invention declarations. Her work hitherto received more than 20,000 citations in Google Scholar base. She supervised 5 PhD and 6 Master students and was a student mentor in the University of Eastern Finland. She was an invited lecturer at several national and international conferences, organiser and lecturer in ISEV educational workshops <https://www.isev.org/blood-ev-workshop>, <https://www.isev.org/bloodev2024>, invited chair in several ISEV and other conferences, founder of Finnish Society of Extracellular and its president 2020-2023. She was awarded Honour from International Society for Extracellular Vesicles as principal organiser of the world first Blood EV Workshop in Helsinki Finland in 2022. She was a member of the editorial board of Journal of Extracellular Vesicles during 2016-2019 and a reviewer in several peer reviewed journals i.e. Stem Cell Research and Development, Cytotherapy, Journal of Extracellular Biology and Biochimica Biophysica Acta.

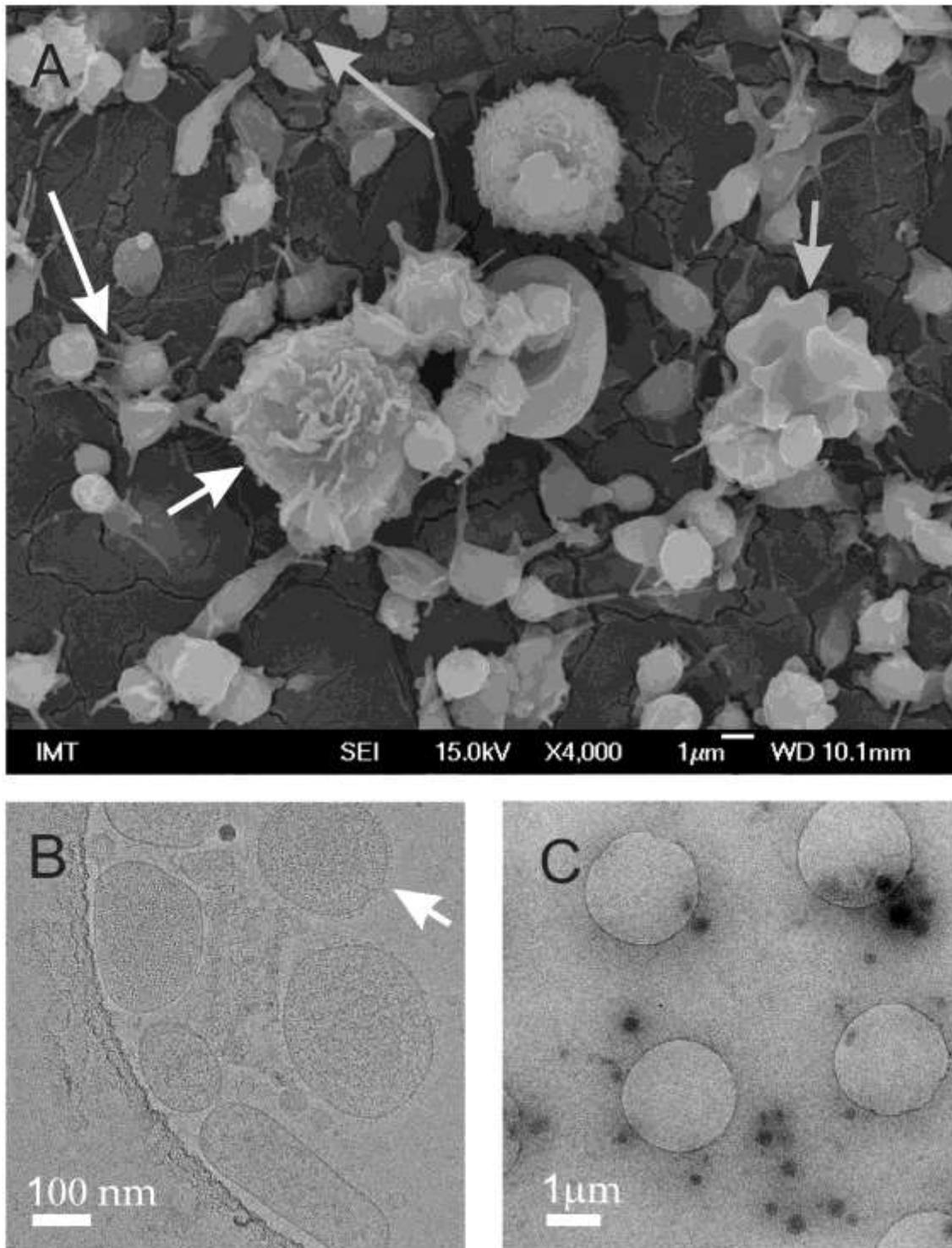
## 3. Extracellular vesicles from blood cells and in blood; The avenue of future therapies by Saara Laitinen

### 2.1. Blood as a liquid tissue

Blood delivers and removes cargo to and from cells, maintains homeostasis, responds to stress and injuries, and is involved in immune and coagulation modulation. It includes cells, gas, hormones, nutrients, and has a vital importance for others tissues and organs. It can be considered as a fluidic tissue and there are similarities and differences between blood and other tissues and organs. Initially considered a simple fluid, blood is now recognized for its complex composition, including red blood cells, platelets, plasma, and components that are not fully understood. Technological advances have shifted the focus of



treatment from whole blood to specific components, thereby improving therapeutic applications. Certain components are yet disregarded or misunderstood, some (e.g. plasma – in practice - blood depleted of erythrocytes (**Figure 1A**)) may even be destroyed during processing.



**Figure 1.** A: Human plasma after 15 hours of fasting (From Božič et al., 2020). White arrow points to a leukocyte, gray arrow points to an erythrocyte, long white arrow points to a platelet and long grey arrow points to an EV. B,C: EVs in isolate from plasma. White arrow points to the corona. A: from Božič et al., 2020), B, C: from Romolo et al., 2022.



The biology of platelets is complex, and they are known to play a role in various physiological and pathological processes, including blood clotting and immune responses. There is a continued relevance of blood products in medicine, despite a decrease in blood-demanding surgical procedures. Blood and plasma are being used for modified antibody-based cancer treatments.

### 3.2. *Extracellular vesicles (EVs) in blood*

EVs are small membrane-enclosed particles released by cells (Jeppesen et al., 2022). They play a crucial role in intercellular communication. EVs in blood derive from blood cells, endothelial cells, but also other types of cells (Nieuwland & Siljander, 2024; Thangaraju et al., 2020). They are shed off from all types of cells to become free to move locally, and if they enter blood, they can travel with it and reach distal cells. Thus, blood EVs are a heterogeneous population of vesicles originating from different cell types, including red blood cells and platelets. The first step in isolation of EVs from blood is preparation of plasma by sedimentation of erythrocytes, most commonly by centrifugation. However, the present protocols of plasma preparation in general disregard the effect of blood processing on the identity, number density, morphology and content of EVs, and these processes are currently being investigated (Ilvonen et al., 2024). The composition of blood EVs is not constant and can vary depending on the cell of origin. The cell-specific origin of EVs is crucial in understanding their behaviour and potential therapeutic applications. **Figure 2** shows EVs isolated from suspension of aged erythrocytes.

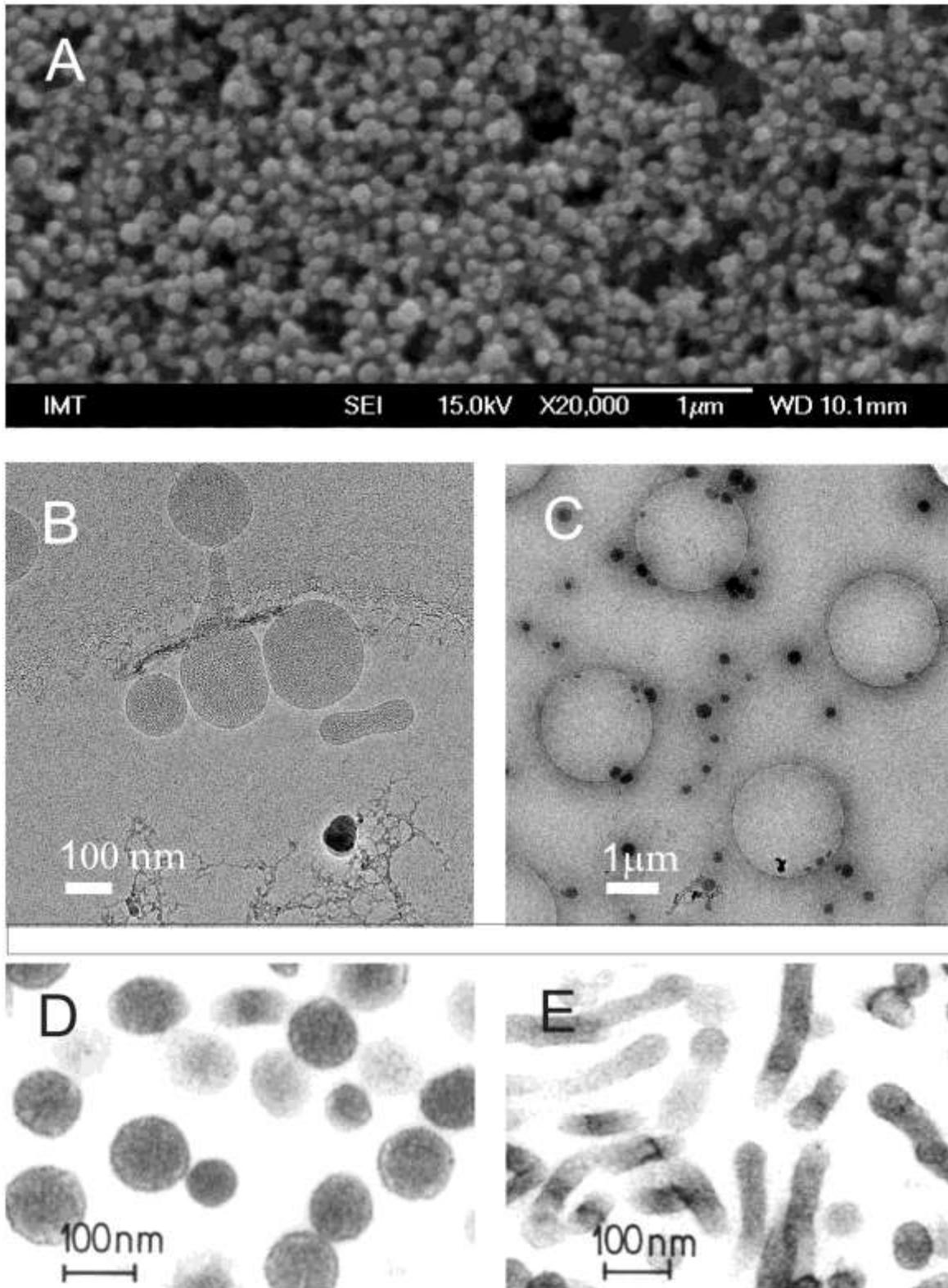
In addition to EVs, there are other types of particles released by cells, known as non-vesicular extracellular particles. These particles vary in abundance and protein concentration. They can be lipid-rich or protein-rich and have distinct compositions and functions. EVs reflect the material of the mother cell. As an example information on the origin can be obtained from the analysis of RAB proteins, revealing how cellular stimuli influence EV composition (Kilpinen et al., 2013). Lipoproteins are abundant in plasma and they can interact with EVs. These particles (initially considered contaminants) may have significant functions, in particular in forming of the vesicle corona (**Figure 1B**). When EVs are released into the bloodstream, they acquire the corona composed of proteins and other components (Tóth et al., 2021; Buzas, 2022).

### 3.3. *EVs as diagnostic vectors*

EVs are considered as potential diagnostic vectors. Research on the interaction of EVs with immune cells, such as monocytes and macrophages, is complex and depends on various factors, including the type of EVs and the time of incubation. These interactions may vary depending on the polarization state of the cell. However, difficulty in identifying specific EVs within a diverse population of particles remains a challenging task often described as finding a "needle in a haystack". Characterization methods are being developed (Koponen et al., 2020). The challenge arises from the diversity of extracellular vesicles and other particles. To overcome this, it is necessary to develop effective targeting moieties and marker combinations. Nevertheless, EVs hold promise as tissue- or cancer-specific diagnostics, provided that appropriate sensitivity thresholds and diagnostic techniques are developed.

### 3.4. *EVs as therapeutic vectors*

Blood-derived EVs have been used therapeutically for many years, particularly in the form of platelet concentrates. These concentrates contain billions of EVs and have been shown to be safe for use in patients. Blood EVs, particularly those derived from red blood cells, have vast potential therapeutic applications depending on the indication, route of administration, and other factors. Potential therapeutic applications of EVs derived from erythrocytes are immune regulation and capillary dilation. Compared to the development of antibody drugs, blood products have historically driven therapeutic advances. Blood-derived EVs provide a natural source of particles that reflect their in vivo environment, offering advantages in terms of safety and functionality. Rapid production of platelet-derived EVs presents opportunities for targeted drug delivery, especially in cancer treatment. It is highly probable that blood and blood cell-derived products will be the next generation of drugs, but the process to accomplish this seems to be protracted.



**Figure 2.** A: Scanning electron micrograph and (B,C): cryogenic transmission electron micrograph of EVs isolated from the suspension of ageing erythrocytes. D and E: Transmission electron micrograph of EVs obtained by isolation from erythrocytes treated with two different detergents, respectively. A: from Jozelj et al. (2022), B and C from Romolo et al. (2022), D and E: from Kralj-Iglič et al. (2020).



There is a lack of universal EV markers, which makes it difficult to distinguish EVs from other particles in the blood. EVs from different blood cells such as erythrocytes and platelets have different properties. Cell-specific isolation of EVs is crucial to increase therapeutic potential.

Blood compatibility is critical for EV-based therapies, as EVs may contain blood group antigens. Research on the interaction of EVs with immune cells, particularly macrophages, is ongoing. These interactions may vary depending on the polarization state of the cell. EVs show potential to target cancer cells, but therapeutic development is still in its early stages. The use of EVs for drug delivery faces obstacles, particularly regarding their ability to penetrate biological barriers such as the blood-brain barrier. Current manufacturing protocols of blood plasma and further plasma medicals (growth factors, immunoglobulins etc) destroy EVs.

### 3.5. *Challenges in EVs harvesting, characterization and applications*

Although EV-based therapies have great potential, challenges in isolation, purification, drug delivery, and diagnostics need to be addressed before they can be widely used in clinical settings. Also, it is necessary to understand the source of EVs and their composition before developing therapeutic strategies. EV cargo (Palviainen et al., 2020) and membrane composition are highly influenced by the blood environment, which complicates their use in standardized therapeutic applications. Blood-derived EVs present challenges due to the unculturable nature of many cells, but this also confers advantages for risk management and post-activation modification.

The EV cargo and membrane composition are dynamic entities influenced by the blood environment, creating both complications and opportunities for diagnostics. The high abundance of non-EV particles in blood adds another level of complication. Current separation methods, particularly those size-based, are inadequate in distinguishing EVs from other blood particles, such as lipoproteins, which are significantly more abundant and overlapping with size. As the development of robust, scalable manufacturing processes is essential to move from small-scale research to large-scale clinical use when using cultured cells/isolators, blood can offer competitive option for production of therapeutic EV source. Moving towards therapy application, regulatory authorities require thorough documentation highlighting the existing expertise within blood services to help manage such complexities. Although robustness and quality control issues may be solved, competition with other drug delivery methods remains and challenge EV research to find the focus where they bring additional value.

### 3.6. *Intertwining EVs with public services*

Blood services possess an extensive history of handling personalized medicine and cell-based therapies, providing a foundation for future advancements. COVID-19 pandemic raised widespread concerns across Europe and globally regarding the security of blood supply. Emerging issues were vaccination strategies and the varying perspectives on the speed of implementation, in the light of historical precedents.

There is a long-standing history of personalized medicine within blood services, exemplified by the understanding of blood groups, tissue typing and their implications for treatment. Moreover, ongoing development of new treatments and services are a key strategic priority. With their experience in addressing regulatory challenges and personalized medicine, blood services are key to the development of cell therapies. It is important to realise that blood-derived EVs have some advantages over EVs derived from various other sources, such as cultured cells or cancerous cells, as blood is readily available in relatively large amounts. Extensive knowledge of blood cell biology, including blood groups, provides a valuable foundation for understanding and working with blood derived EVs.

The Finnish Red Cross Blood Service is centrally responsible for the blood supply throughout Finland, distinct from the more decentralized models in many countries. This centralized structure presents many advantages to implement novel treatments and services nationwide, but it has demanded to develop its logistics to serve entire country.



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## Review

# The Mammary Gland: Anatomy, Histology, Pathology and Post-Surgery Physiotherapy Management

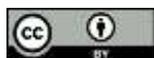
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**Abstract:**

The mammary gland is a highly specialized organ dedicated to milk production, composed of glandular, connective, and adipose tissues. It undergoes dynamic structural changes throughout life, influenced by hormonal cycles essential for reproduction and lactation. Despite its vital physiological role, the mammary gland is susceptible to pathological conditions, most notably breast carcinoma, which remains the leading cause of cancer-related deaths among women. This article provides an overview of the anatomy, histology, pathology, and physiotherapy rehabilitation strategies related to the mammary gland. A literature review was conducted drawing from peer-reviewed articles, textbooks, and guidelines to examine mammary gland anatomy, histology, breast carcinoma, and physiotherapy approaches following surgery. The lymphatic system, particularly the axillary lymph nodes, plays a crucial role in immune defense and serves as the primary route for metastatic spread in breast cancer, making it a key diagnostic and prognostic marker. Breast carcinoma can be benign, non-invasive malignant, or invasive malignant. Invasive carcinomas, including ductal and lobular carcinoma, are the most aggressive and frequently metastasize through lymphatic pathways. Post-surgical physiotherapy plays a vital role in managing complications like lymphedema, improving upper limb mobility, and supporting psychological well-being. Rehabilitation involves methods like manual lymphatic drainage, compression therapy, and advanced treatments, including low-level laser therapy, all aimed at accelerating recovery and enhancing quality of life. In Slovenia, physiotherapy protocols emphasize early patient education, gradual exercise progression, and lymphedema prevention. Personalized strategies integrating anatomical and pathological knowledge significantly enhance recovery outcomes and quality of life for breast cancer patients.

**Keywords:** Mammary gland anatomy and histology; Lymphatic system; Breast carcinoma; Breast cancer rehabilitation; Physiotherapy management.

## 1. Introduction

The mammary gland is a sophisticated anatomical structure composed of glandular and adipose tissue, supported by fibrous connective tissue and positioned over the pectoral muscles of the chest wall. Extending from the second to seventh ribs, it is intricately designed with a network of blood vessels, lymphatic pathways, and sensory nerves, all of which contribute to its structural integrity, functional capacity, and physiological significance. A thorough understanding of its anatomy forms the basis for comprehending its role in health and disease. From a histological perspective, the mammary gland's complexity is further highlighted by its dynamic nature. Throughout an individual's life, hormonal cycles drive significant structural changes, enabling its essential role in reproduction and lactation. These adaptations not only support milk production but also underscore the gland's susceptibility to various pathological conditions.

An essential part of the mammary gland's physiology is its connection to the lymphatic system. This network, comprising lymphatic vessels, nodes, and major ducts, is critical for maintaining tissue–fluid balance, immune defense, and the clearance of interstitial fluid (Leong et al., 2022; Schuenke et al., 2010). Lymph nodes, such as the axillary nodes, play a pivotal role as filtering stations, cleansing lymph of pathogens and debris. Furthermore, the lymphatic system is a key pathway for the metastatic spread of cancer cells, with lymph nodes serving as important indicators of disease progression in breast cancer patients (Null et al., 2023; Natale et al., 2021).

One of the most significant pathological challenges associated with the mammary gland is breast cancer, which remains the most frequently diagnosed cancer worldwide. Accounting for 25% of all diagnosed cancers, it predominantly affects women, although men are also occasionally diagnosed with the disease (Tosello et al., 2018). Numerous risk factors, including gender, age, obesity, alcohol consumption, sedentary lifestyle, hormone exposure, radiation, family history, and genetic predisposition, contribute to its development (WHO, 2024). While the incidence of breast cancer has been rising, advances in early detection and treatment have significantly improved survival rates, particularly for localized stages of the disease (Katsura et al., 2022). Given the rising prevalence of breast cancer and the physical and emotional challenges faced by patients post-surgery, the role of targeted physiotherapy rehabilitation programs is increasingly recognized. These programs not only focus on restoring physical strength and upper limb function but also address psychological well-being, playing a critical role in enhancing overall recovery and quality of life after breast surgery.

## 2. Design of the Review

A literature review was conducted, selecting articles published between 1992 (Stark et al., 1992) and 2024 (WHO, 2024; Yang et al., 2024). The review focused on publications that described or examined the anatomical features and histological changes of the mammary gland, breast carcinoma and various physiotherapy approaches for managing patients after breast cancer surgery. Studies published in English and Slovene were included to ensure comprehensive coverage.

The reviewed literature encompassed a wide range of sources, including peer-reviewed scientific articles, academic textbooks, institutional guidelines, dissertations, and credible online resources. Peer-reviewed articles formed the backbone of the analysis, providing evidence-based insights into related topics, while systematic reviews and meta-analyses offered a synthesis of current research. Foundational knowledge was derived from academic textbooks and atlases, such as Schuenke et al. (2010) and Hayes & Nguyen (2014), which detailed anatomical and histological aspects of the mammary gland. Institutional guidelines, including those by the Slovenian Institute of Oncology (Onkološki inštitut, 2019), were integral in describing standardized rehabilitation approaches.

## 3. Breast Anatomy

The breast is a complex anatomical structure primarily composed of glandular and adipose tissue, supported by connective tissues and overlying the pectoral muscles of the chest wall (Dahmane and Cor, 2001). Its primary function is lactation, but the breast also holds

significant importance in physical, psychological, and sexual contexts (Bazira et al., 2022; Khan et al., 2025).

Women and men both have breasts (Torre et al., 2016). The breast of an adult woman typically weighs between 150 and 200 grams. It is located on the anterior chest wall, extending from the second or third rib to the sixth or seventh costal cartilage, medially reaching the sternal edge and laterally extending to the mid-axillary line. The breast is situated on the pectoralis major muscle, partially on the serratus anterior muscle, and on the external oblique abdominal muscle. The pectoralis minor muscle, located beneath the pectoralis major, serves as an important anatomical landmark for dividing the levels of lymph node drainage (Marolt Mušič et al., 2004).

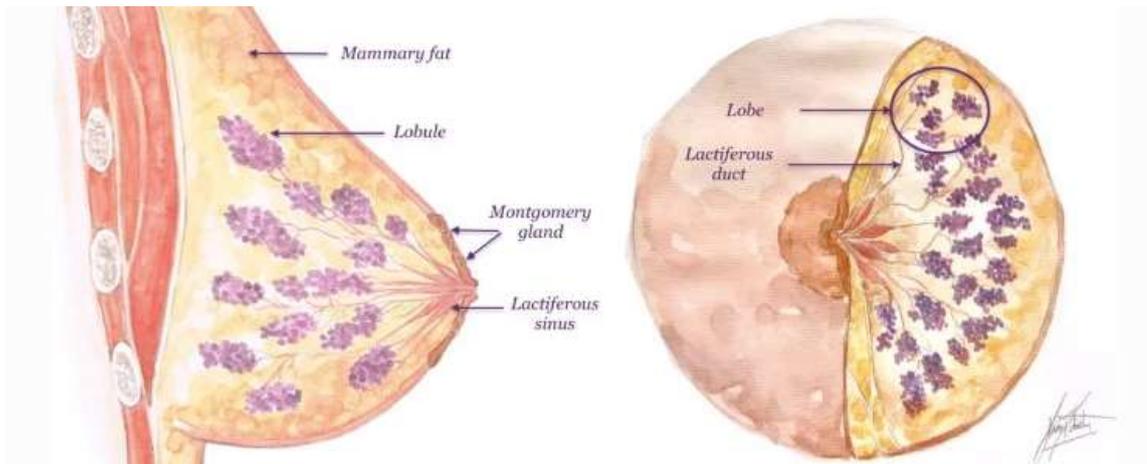
The glandular tissue of the breast is covered by fascia, with its posterior surface being slightly concave to conform to the underlying muscles. (Marolt Mušič et al., 2004). Fatty tissue, known as adipose tissue, makes up the breasts (Aronson et al., 2000). The adipose tissue in the breasts is intertwined with a network of nerves, blood vessels, lymphatic vessels, lymph nodes, and also consists of fibrous connective tissue and ligaments (Thomsen & Tatman, 1998).

The sensory innervation of the breast arises from the anterior and lateral cutaneous branches of the intercostal nerves (fourth to sixth thoracic levels). This innervation not only mediates sensation but also plays a role in the hormonal regulation of lactation and sexual responsiveness (Rivard et al., 2023). The vascular supply of the breast is extensive, originating primarily from the internal thoracic artery, the lateral thoracic artery, and branches of the thoracoacromial artery. Venous drainage follows a similar pattern, emptying into the axillary and internal thoracic veins (Bazira et al., 2022).

The breast's lymphatic drainage is critical for immune defense and has significant implications for the metastatic spread of breast cancer. Approximately 75% of lymphatic drainage is directed toward the axillary lymph nodes, with additional pathways leading to the parasternal and supraclavicular nodes (Khan et al., 2025). Breasts vary in shape and size, with one breast often being slightly smaller than the other, and nipples also show a wide range of variation (Brayboy et al., 2017). The epidermis of the areola and nipple is highly pigmented and slightly wrinkled, while the skin of the nipple contains numerous apocrine and sebaceous glands, along with some small hairs. At the base of the nipple, milk ducts converge and expand into milk sinuses. These ducts facilitate the transport of milk to the nipples (Hassiotou & Geddes, 2013). Supporting these structures are fibrous tissues, including Cooper's ligaments, which help maintain the breast's shape and position (Khan et al., 2025). Female breasts typically contain more glandular tissue compared to male breasts (Stark et al., 1992). Female breasts are composed of 12–20 lobes, which are divided into smaller lobules (Tanis et al., 2001). These lobes and lobules are interconnected by 15–25 milk ducts. Female breasts are adapted to provide the best possible nourishment for babies and to offer sexual satisfaction to the woman. They are glandular organs that respond strongly to hormonal changes in the body (Jagannathan & Sharma, 2017).

#### 4. Histology of the Breast Tissue

The breast (*glandula mammaria*) is a highly specialized organ composed of glandular, connective, and adipose tissues, with its histological architecture undergoing significant changes throughout life and uniquely designed for milk production. The glandular tissue has a tubular-alveolar structure which is organized into 15 to 20 lobes (*lobus mammae*). These alveoli are lined by a single layer of cuboidal epithelial cells, supported by contractile myoepithelial cells and a basement membrane, which aids in milk ejection. Lobes are arranged around the nipple (*papilla mammae*) where the epithelium transitions from simple cuboidal in the smaller ducts to stratified cuboidal and then to stratified squamous epithelium near the nipple (Baumgartner, 2018). Each lobe is drained by a lactiferous duct (*ductulus lactiferus*) and each lactiferous duct expands into a lactiferous sinus (*sinus lactiferus*), which narrows as it approaches the nipple and opens with a small pore (**Figure 1**). Within each lobe, smaller subdivisions called lobules (*lobulus mammae*) contain terminal duct lobular units (TDLUs), the primary structures responsible for milk production. (Takač, 1996; Hayes & Nguyen, 2014). Dense connective stroma surrounds the glandular structures, interspersed with adipose tissue that contributes to the breast's size and shape.



**Figure 1:** Anatomical structure of the breast (*glandula mammaria*), highlighting its histological organization. On the left, a sagittal section of the breast shows the mammary fat, lobules, lactiferous sinuses, and Montgomery glands. On the right, a magnified view of a lobe reveals the lobes, smaller lobules, and lactiferous ducts, which converge into lactiferous sinuses. The breast's structure is specialized for milk production and secretion, supported by alveoli lined with glandular epithelium and contractile myoepithelial cells, which facilitate milk ejection.

The size and composition of the breast tissue can vary between individuals and even within different regions of the same breast, often influenced by age, hormonal status, and functional activity (Hayes & Nguyen, 2014). The breast undergoes dynamic changes throughout a woman's life, including ductal growth and alveolar proliferation during puberty, rapid expansion and secretion during pregnancy, and eventual regression during menopause. During pregnancy, the ductal system grows extensively, forming new terminal ducts and alveoli, which become fully differentiated by late gestation to facilitate milk secretion. Following menopause, glandular tissue is replaced by fibrous and fatty tissue as hormonal stimulation decreases (Dahmane & Cör, 2001). These functional changes are influenced by hormonal cycles and are essential for the breast's role in lactation and reproduction (Hayes & Nguyen, 2014). Understanding these histological variations is crucial for diagnosing and managing breast diseases.

## 5. Lymphatic system

The human lymphatic system, comprising lymphatic vessels, lymph nodes, and lymphoid organs such as the tonsils, thymus gland, and spleen, plays a critical role in maintaining tissue–fluid balance and body homeostasis (Leong et al., 2022; Natale et al., 2021). Functioning parallel to the venous system, it has primary responsibilities that include clearing interstitial fluid and substances that cannot be reabsorbed into venous capillaries, transporting food lipids (e.g., chylomicrons absorbed in the intestine), and returning lymphocytes from lymphoid organs to the bloodstream (Schuenke et al., 2010).

The lymphatic vascular system consists of lymphatic capillaries, vessels interspersed with lymph nodes, and major trunks such as the thoracic duct and right lymphatic duct. Peripheral lymphatic capillaries, which begin as blind-ended vessels, collect interstitial fluid and transport it through lymphatic vessels and nodes to major lymphatic trunks. These trunks return lymph to the venous system at the junctions of the subclavian and internal jugular veins (Schuenke et al., 2010). Lymph nodes, small bean-shaped structures situated along lymphatic vessels, act as filtering stations and secondary immune centers. Afferent lymphatic vessels bring lymph into the nodes, where macrophages and lymphocytes cleanse it of pathogens and debris. The filtered lymph then exits through efferent vessels, continuing its journey toward major ducts and the venous system (Null et al., 2023).

The axillary lymph nodes (**Figure 2**) serve to drain the entire upper limb, the breast and the trunk above the umbilicus and are categorized into groups (Khan et al., 2025):

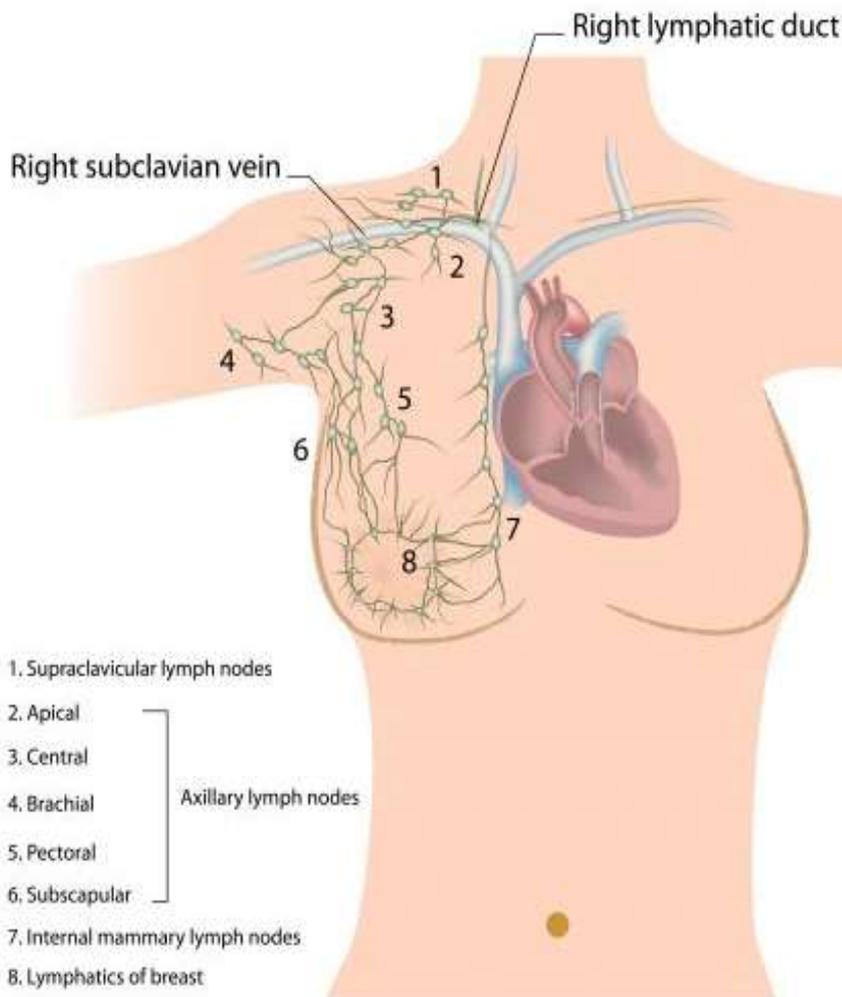
- Anterior (pectoral) nodes are located along the lower border of the pectoralis minor, behind the pectoralis major muscle. They receive lymph vessels from the lateral quadrants of the breast and superficial vessels from the anterolateral abdominal wall above the level of the umbilicus. There are usually 4-5 large nodes in this group.
- Posterior (subscapular) nodes, consisting of 6-7 nodes, lie in front of the subscapularis muscle and receive superficial lymph vessels from the posterior trunk down to the level of the iliac crests.
- Lateral nodes, comprising 4-6 nodes lie along the medial side of the axillary vein and receive most of the lymph vessels of the upper limb, except for superficial vessels draining the lateral side (see infraclavicular nodes below).
- Central nodes, located in the center of the axilla within the axillary fat, receive lymph from the anterior, posterior, and lateral groups and typically consist of 3-4 nodes.
- Apical nodes, also referred to as the subclavicular group, are found at the apex of the axilla near the lateral border of the first rib. These nodes collect the efferent lymphatic drainage from all the other axillary nodes.
- Infraclavicular (deltopectoral) nodes are not strictly axillary nodes, since they are located outside the axilla. They lie in the groove between the deltoid and pectoralis major muscles and receive superficial lymph vessels from the lateral side of the hand, forearm, and arm.

The lymphatic system also plays a pivotal role in disease processes, particularly cancer. Unlike the cardiovascular system, the lymphatic network consists of nearly invisible, fragile vessels that transport clear, colorless lymph. This system is the main pathway for the spread of cancer cells, with malignant cells colonizing lymph nodes and distant organs, leading to a poorer prognosis in breast cancer patients. Mapping lymphatic flow patterns could enable the prediction of tumor spread based on the primary tumor site. (Leong et al., 2022; Natale et al., 2021).

## 6. Breast Carcinoma

Breast carcinoma is the leading cause of cancer related deaths among women (Keen & Davidson, 2003). According to WHO (2024) it is believed that breast cancer caused approximately 670000 deaths globally in 2022. Only 0,5–1 % of breast cancer occurs in men, other 99 % cases occur in women. The strongest risk factor is gender, other risk factors are: age, obesity, increased alcohol consumption, a sedentary lifestyle, exogenous hormone exposure, including contraceptive pills and hormone replacement therapy, radiation exposure, family history of breast cancer and genetic predispositions (mutation of genes - BRCA1 and BRCA2 and others) etc (WHO, 2024). Although survival rates have significantly improved over the past two decades, the incidence of this disease continues to rise worldwide. The incidence increases with age, with over 80% of cases occurring in women over 50 (Nolan et al., 2023).

Breast cancer often presents as a painless lump in the breast or armpit. A lump is usually a palpable structure and can be of any type. Additional signs (**Figure 3**) may present as breast swelling, changes in shape or size of the breast as well as skin changes such as erythema, pitting, an "orange peel" texture (peau d'orange) and ulceration. Alterations to the nipple, such as inversion, skin changes, or discharge, may also occur (Katsura et al., 2022; Mohallem Fonseca et al., 2019). These breast lumps on the other hand can be a sign of inflammation, hyperplastic proliferation or just fluid accumulation. However neoplastic proliferations can be present without lump formation (Cserni, 2020).



**Figure 2:** The lymphatic drainage of the breast, highlighting the lymphatic vessels and lymph nodes in the axillary region, involved in maintaining tissue-fluid balance and immune defense (Comans, 2019). These include the pectoral, central, subscapular, brachial, and apical groups, which collectively drain lymph from the breast, upper limb, and adjacent chest wall. The supraclavicular lymph nodes and internal mammary lymph nodes are also shown, representing additional drainage pathways. The right lymphatic duct connects this system to the right subclavian vein, demonstrating the integration of lymphatic drainage into the venous circulation

Breast tumors can be divided into benign and malignant. The main difference between benign and malignant tumors is that benign tumor cells do not spread, do not metastasize to other parts of the body, and rarely need to be treated. They can cause uncomfortable symptoms or changes in the appearance of the breast. Benign breast tumors include fibroadenoma, hamartoma, lipoma, and intraductal papilloma. Breast carcinomas can be classified according to histological grade or histological type. Histological grade assesses the degree of differentiation (tubule formation and nuclear pleomorphism), the proliferative activity of a tumour and mirrors its aggressiveness. The grades of breast carcinomas range from I. which is most similar to normal healthy cells, to III. which is the most different from normal cells and most aggressive. Histological type refers to the growth pattern of the carcinoma. Among the most common are ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC). DCIS is a non-invasive malignant carcinoma that originates in the lactiferous ducts and remains confined to the ductal system without spreading to surrounding tissues. It is considered an early form of breast cancer with high treatment success rates. IDC, on the other hand, arises from DCIS when the cancer cells invade surrounding healthy breast tissue. It is the most prevalent type of breast cancer and is capable of metastasizing to other parts of the body. Another significant type is invasive lobular carcinoma (ILC), which originates in the lobules. ILC tends to be bilateral and multicentric, often spreading to bones, ovaries, or serous cavities. Less common types of

breast cancer include inflammatory breast cancer, which presents with swelling and redness due to lymphatic blockage; tubular breast cancer, characterized by its tubular structure under microscopic examination; and colloid breast cancer, which produces mucin. (Weigelt et al., 2010). Breast cancer stage is determined by tumor size, nodal involvement, metastases and the presence of specific biomarkers such as estrogen receptors, progesterone receptors and ERBB2 receptor. DCIS is stage 0, noninvasive breast cancer. Early invasive cancer describes stages I, IIa, and IIb, and locally advanced cancer describes stages IIIa, IIIb, and IIIc. All of these stages of breast cancer are nonmetastatic. Stage IV is metastatic breast cancer. Breast cancer which do not express any of the specific biomarkers are referred to as triple-negative. Breast cancer is treated with preoperative and postoperative systemic therapies that include chemotherapy, endocrine therapies, immunotherapy with monoclonal antibodies directed at tumor receptors, surgery and radiation (Trayes & Cokenakes, 2021).



**Figure 3:** Common signs and symptoms associated with breast cancer (*Breast Cancer Symptoms and Signs*, n.d.). These symptoms highlight potential abnormalities that may warrant further medical investigation, as they can be indicative of underlying pathological processes, such as inflammation, fluid accumulation, or neoplastic proliferation.

## 7. Physical therapy approach after breast cancer surgery

Rehabilitation after breast cancer surgery is crucial for improving physical function, managing pain, preventing complications like lymphedema, addressing psychological well-being, and enhancing overall quality of life (Donahue et al., 2023). Breast cancer surgery can involve procedures that significantly impact the body (De Groef et al., 2015). Rehabilitation helps manage and improve physical function, flexibility, and strength that may be lost due to surgery (De Groef et al., 2015).

The primary focus of post-surgery rehabilitation is improvement of upper limb function, because many patients experience reduced mobility and function (De Groef et al., 2015). Addressing and managing pain effectively (Olsson Möller et al., 2019) and prevention and management of post-operative complications are primary rehabilitation goals especially after lymph node removal (Davies et al., 2020). Beyond physical recovery, rehabilitation must address the psychological impact of breast cancer (Olsson Möller et al., 2019).

Rehabilitation programs are designed to improve muscle function and range of motion, incorporating both active and passive stretching exercises, and strengthening routines for the shoulder girdle muscles (De Groef et al., 2015). Complete decongestive therapy, which

uses manual lymphatic drainage, the use of compression bandages, and skin care are part of the standardized rehabilitation protocol when managing lymphedema (Donahue et al., 2023). Kinesiology tapes can also be used (Yang et al., 2024). Physical Agents such as TENS and cryotherapy may help with pain reduction. Whereas advanced treatments such as low-level laser therapy (Davies et al., 2020) and hydrotherapy (Wang et al., 2022) play a significant role in reducing pain, enhancing tissue healing and lowering inflammation as well. Rehabilitation programs are tailored to meet the physical and emotional needs of the patient, ensuring they regain their strength, function and confidence to lead a more fulfilling life (De Groef et al., 2015).

## 8. Discussion

Breast anatomy and its clinical implications are particularly significant in the context of breast cancer, one of the most common malignancies affecting women worldwide. The mammary gland is comprised of glandular and adipose tissues and supported by connective structures. It is richly vascularized by branches of the internal thoracic, lateral thoracic, and thoracoacromial arteries, with venous drainage primarily directed into axillary and internal thoracic veins (NCBI, 2020; Cleveland Clinic, n.d.). The lymphatic drainage system is of paramount importance in the development of breast cancer, as the axillary lymph nodes are often the first sites of metastasis. In breast cancer, axillary lymph nodes are crucial for drainage from the upper limb and breast, as approximately 75% of lymphatic drainage flows into these nodes (Leong et al., 2022). The lymphatic system is the main pathway for cancer cell spread, making lymphatic mapping and evaluation essential for predicting tumour spread and prognosis. Early detection of metastasis can significantly impact treatment and outcomes in breast cancer patients (Khan et al., 2025).

Histologically, the breast undergoes dynamic remodelling influenced by hormonal changes throughout life, with notable changes during puberty, pregnancy, and menopause. The terminal duct lobular units, essential for lactation, are also the primary sites for the origin of most breast carcinomas, underscoring the need for a detailed understanding of breast histology to guide diagnosis and treatment (Hayes & Nguyen, 2014). Recognizing these changes is essential for understanding normal physiology and identifying pathological conditions, aiding in better diagnosis and treatment of breast-related diseases (Leong et al., 2022). Furthermore, correct diagnosis and treatment, aided with physical therapy is essential for patient-focused rehabilitation (De Groef et al., 2015).

Although the incidence of breast cancer rises, survival rates have been improving. In 2020 the World Health Organization reported 685000 deaths globally and by 2022 these rates had decreased further (WHO, 2024). This decline may be attributed to heightened public awareness regarding healthy lifestyle practices, such as reducing alcohol consumption, alongside significant improvements in early diagnostic measures, rapid treatment protocols, and prompt medical interventions (Nolan et al., 2023). Post-operative physical therapy is an integral component of breast cancer care, aiming to restore function, prevent complications, and improve the quality of life. Programs focus on improving upper limb mobility and strength through active and passive exercises, alongside specialized treatments like manual lymphatic drainage, compression therapy, and advanced modalities such as low-level laser therapy and hydrotherapy (Donahue et al., 2023; Davies et al., 2020). In Slovenia, the standardized post-operative rehabilitation approach is structured and phased. Early post-operative physical therapy focuses on education, proper posture, and simple shoulder exercises, progressing to advanced active exercises and scar massage after suture removal. Health education, especially for lymphedema prevention, is emphasized from the outset. Outpatient therapy additionally ensures continuity of care (Institute of Oncology, 2019). Compared to international practices, the Slovenian protocol places a stronger emphasis on early education and the gradual progression of activities tailored to individual needs. While global protocols often recommend earlier, more aggressive mobilization and manual therapy, the Slovenian approach favours a more cautious, stepwise methodology to minimize complications like lymphedema and ensure patient comfort (Leong et al., 2022). This structured approach demonstrates the importance of integrating anatomical and histological knowledge with patient-centred therapeutic strategies to optimize outcomes.

## 9. Conclusions

The mammary gland is a complex organ consisting of glandular, adipose, and connective tissues, essential for reproductive and physiological functions. Its dynamic histology, shaped by hormonal changes, increases its vulnerability to diseases such as breast cancer, a persistent global health priority. Breast cancer frequently originates in the terminal duct lobular units, emphasizing the importance of detailed anatomical and histological knowledge for accurate diagnosis and treatment. Advances in understanding the lymphatic system have enhanced the prediction of cancer metastasis, a critical factor in prognosis. Although progress has been made in early detection and treatment, breast cancer remains a significant health challenge, with rising incidence rates underscoring the need for ongoing research. Post-surgical physiotherapy plays a vital role in recovery, addressing both physical impairments and emotional well-being.

**Conflicts of Interest:** The authors declare no conflict of interest.

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*Review*

# Role of Extracellular Vesicles in Breast Cancer, its Diagnostics and Treatment

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**Abstract:**

While the risk factors of breast cancer have been extensively studied and recorded, the understanding of the microscopic and nanoscopic mechanisms causing the disease is not yet satisfactory. Recent research in cell biology points to sub-micron sized extracellular vesicles (EVs) which are by now acknowledged to deliver cargo to cells and thereby mediate interaction between them. Investigation of EVs is challenging, as presently there are no golden standard methods for their isolation from bodily fluids or tissues, and their characterization. They are tiny (nano-sized) particles with dynamic identity that require development of new technologically advanced methods. This contribution presents a brief survey of the evidences on various facts that are being collected on breast cancer EVs.

**Keywords:** Extracellular vesicles; Extracellular vesicles in cancer; Extracellular vesicles in breast cancer; Extracellular particles; Extracellular particles in cancer; Extracellular particles in breast cancer

## 1. Etiology of breast cancer

Breast cancer is the most often diagnosed and the most prevalent cancer of all types (Luo et al., 2022). Although there are reports on different risk factors, the etiology of cancer is not known. Incidence rate increases with age and saturates after about 55 years of age (Luo et al., 2022). It was found that lifestyle increases the risk for breast cancer incidence, e.g. smoking in premenopausal women (Peñalver-Argüeso et al., 2023), alcohol intake (McDonald et al., 2013), higher intake of sugar (Farvid et al., 2021) and red meat (Lo et al., 2020). Some chemicals were connected to higher incidence of breast cancer: pollutants (White et al., 2023), food additives (Sellem et al., 2024), heavy metals (Romaniuk et al., 2017), radiation (Preston et al., 2016), drug use disorder (Dahlman et al., 2021), psychological factors (Greer & Morris, 1975), lack of physical activity, and obesity (Hardefeldt et al., 2018). Genetic factors play a significant role in many cases of breast cancer, particularly mutations in the BRCA1 and BRCA2 genes which are associated with a high risk of developing the disease (Mehrgou et al., 2016). Additionally, various other genetic alterations contributing to breast cancer have been identified (Pal et al., 2024). The reported risk factors indicate the link of cancer incidence with hormonal status (Henderson & Feigelson, 2000). Hormonal factors, especially prolonged exposure to estrogen, are also crucial in the development of breast cancer (Al-Shami et al., 2023). Estrogens promote the growth of breast tissue, which may contribute to the development of cancerous cells, particularly in cases of hormonal imbalance or excessive estrogen production (Yaghjian & Colditz, 2011). Early menarche (Harris et al., 2024; Lopes et al., 2024), late menopause (Lopes et al., 2024), and hormone replacement therapy (Vinogradova et al., 2020) are all risk factors that increase the likelihood of developing breast cancer.

## 2. Extracellular vesicles (EVs)

Extracellular vesicles (EVs) are submicron membrane-bound structures released by all cell types into the extracellular space (Yanez-Mo et al., 2015). They are classified into subtypes based on their biogenesis and size, including exosomes, microvesicles (or ectosomes), and apoptotic bodies (Yanez Mo et al., 2015).

EVs have emerged as essential mediators of intercellular communication, carrying diverse molecular cargo, including proteins, lipids, RNA, and DNA (Welsh et al., 2024). This cargo allows EVs to influence a variety of biological processes (Yanez Mo et al., 2015; Welsh et al., 2024). EVs are involved in various disease processes, particularly cancer. They are present in various biological fluids and hold great potential as biomarkers for early cancer detection, disease progression monitoring, and treatment response evaluation (Póvoa & Rodrigues, 2022; Bamanakar et al., 2023). Being crucial for cell-to-cell signaling, they influence tumor growth, metastasis, and immune responses, thus opening new possibilities for diagnosis and therapy (Chang et al., 2021). In breast cancer, EVs hold promise as non-invasive biomarkers detectable in blood and other body fluids for early diagnosis and monitoring (Xu et al., 2024). Ongoing research explores their use for therapeutic applications such as targeted drug delivery due to their ability to transport therapeutic agents directly to tumor cells.

## 3. Mechanisms of EV formation

### 3.1. Endosomal Pathway (Exosome Formation)

Exosomes, a subtype of EVs, are generated through the Endosomal Sorting Complexes Required for Transport (ESCRT) - dependent and -independent mechanisms (Teng & Fussenegger, 2020). Initially, the plasma membrane invaginates, forming early endosomes that mature into late endosomes, also known as multivesicular bodies (MVBs) (Yanez Mo et al., 2015). The inward budding of the endosomal membrane leads to the formation of intraluminal vesicles (ILVs) within MVBs. These ILVs eventually fuse with the plasma membrane, releasing exosomes into the extracellular space (Yanez Mo et al., 2015). The ESCRT machinery plays a critical role in sorting and packaging cargo into ILVs, while the tetraspanin family of proteins, such as CD63, also contribute to this process in an ESCRT-independent manner (Teng & Fussenegger, 2020).

### 3.2. Budding from the Plasma Membrane (Microvesicle Formation)

Microvesicles, also called ectosomes, are formed through direct outward budding of the plasma membrane (Kralj-Iglič et al., 2020). This process is driven by the reorganization of the actin cytoskeleton and the activation of specific lipid signaling pathways (Romer et al., 2010). The local accumulation of phosphatidylserine (PS) on the inner leaflet of the membrane and the interaction with various membrane-binding proteins promote membrane curvature, leading to the formation and release of microvesicles (Record et al., 2018). Unlike exosomes, microvesicles do not involve endosomal intermediates but are directly shed from the cell surface.

### 3.3 Apoptotic Body Formation

Apoptotic bodies are larger EVs formed during the programmed cell death (apoptosis) process (Yanez Mo et al., 2015, Kralj-Iglic et al., 2020). As cells undergo apoptosis, the plasma membrane begins to bleb, and fragments of the dying cell, including cytoplasm, organelles, and nuclear fragments, are packaged into apoptotic bodies. These larger vesicles are then released into the extracellular space, and they typically serve to eliminate cellular debris in an organized manner, facilitating immune system clearance (Boada - Romero et al., 2020).

### 3.4 Regulatory Factors

The mechanisms of EV formation are complex and involve multiple pathways that enable cells to release distinct types of vesicles into the extracellular space. Exosome formation through the endosomal pathway, microvesicle shedding from the plasma membrane, and apoptotic body formation during cell death are all critical processes in cellular communication. The formation and release of EVs are regulated by various factors, including cellular stress, environmental signals, and disease states. Key signaling pathways, such as those involving RABS (small GTPases), phosphoinositides, and tetraspanins, play critical roles in regulating vesicle biogenesis (Rädler et al., 2023). Additionally, cellular conditions like hypoxia, senescence, oncogene activation, oxidative stress (Chiaradia et al., 2021), inflammation (Ammirata et al., 2024) can influence the quantity and cargo of EVs, thereby modulating their functions in both physiological and pathological contexts.

In contrast to exosomes, microvesicles form through a different process that involves direct outward of the plasma membrane (Kralj-Iglič et al., 2020). This pathway, often referred as ectosome formation, is driven by the rearrangement of the cytoskeleton, specifically actin filaments, and the activation of various lipid signaling pathways (Meldolesi, 2018). These processes induce membrane curvature, leading to the shedding of vesicles from the cell surface. Microvesicles are typically larger than exosomes, with diameters ranging from 100 to 1000 nm.

## 3. Methods of EV isolation from bodily fluids

**Ultracentrifugation:** This standard technique uses high-speed centrifugation to separate EVs based on their density. It is efficient but time-consuming and requires precise optimization of speed and duration. High centrifugal forces can also damage EVs, affecting their integrity and functionality (Božič et al., 2019). **Size-Exclusion Chromatography (SEC):** SEC separates EVs by size as they pass through a porous matrix, offering higher purity compared to ultracentrifugation, however, it is slower and may require optimization for specific sample types (Clos-Sansalvador et al., 2022). **Density Gradient Centrifugation (DGC):** DGC separates particles through a gradient of different densities, providing high purity, particularly in complex samples, however, it is complex, time-consuming, and requires careful handling (Clos-Sansalvador et al., 2022). Additional techniques like ultrafiltration, immunoaffinity capture (Welsh et al., 2024) and microfluidic analysis (Chen et al., 2024) are emerging for EV isolation from smaller samples.

## 4. Methods of EV isolation from tissues

Isolating EVs from tissues is complex due to the challenge of extracting them from the extracellular matrix (Crescitelli et al., 2021). Successful isolation has been reported from

various tissues including adipose tissue (Sabio and Crewe, 2023), tumors (Swatler et al., 2024), placenta (Zabel et al., 2020), and brain (Metamoros-Angles et al., 2024). In the central nervous system (CNS), EVs released by nerve cells play roles in both normal and pathological processes (Gassama et al., 2021). Protocols for isolating EVs from tissue interstitial fluids have been developed only recently (Guerrero-Alba et al., 2024). The MISEV2023 guidelines, updated by the International Society for Extracellular Vesicles (ISEV) (Welsh et al., 2024), offer standardized practices for isolating and characterizing EVs from solid tissues. These guidelines emphasize: Clarification of terminology to ensure consistent use of EV definitions; Experimental design to ensure reliable and reproducible results; Method flexibility, allowing researchers to select methods suitable for their research needs. EVs from tissues are typically separated based on biophysical properties like size, density, and surface composition (Welsh et al., 2024).

### 5. Methods of EV characterization

Western blotting, Nanoparticle Tracking Analysis (NTA), and Electron Microscopy (EM) are commonly used to analyze EV composition, number density, and morphology, respectively (Welsh et al., 2024). Despite these challenges, EVs show promise for identifying new biomarkers that could enable early cancer detection (Póvoa & Rodrigues, 2022; Möller & Salomon, 2023). Advanced technologies, such as mass spectrometry and sequencing, enable the identification of potential biomarkers and the improvement of diagnostic tools (Pocsfalvi et al., 2016). Advances in techniques such as nonlinear optical microscopy, which uses endogenous contrasts, have enhanced the ability to analyze EVs' biochemical and functional properties (Sorrells et al., 2024). Advanced deconvolution methods can be used to assess the EV transcriptome and compare expression profiles with different cell types (Larsen et al., 2024). Cancer cell-derived EVs carry molecules like messenger RNA and micro RNA, which affect signaling pathways and protein expression in target cells, supporting tumor growth and spread (They et al., 2018). However, quantifying EVs remains challenging, as many methods rely on indirect markers or particle counts, which may not always be specific to EVs (Atlantis Bioscience, 2024).

### 6. Types and treatments of breast cancer

Carcinomas are tumors that start in the epithelial cells that line organs and tissues throughout the body. According to the type of cells in the breast that become cancer cells, we distinguish ductal carcinoma (originating in the milk ducts) and lobular carcinoma (originating in the glands in the breast that make milk). If the cancer remains in the duct, it is called ductal carcinoma in situ; if it expanded in other breast tissue, it is called invasive or infiltrating breast cancer. Cancer cells carry estrogen (ER) and progesterone receptors (PR) and can be accordingly stimulated by the hormones (HR) (Orrantia-Borunda et al., 2022). Some have increased quantities of the human epidermal growth factor receptor-2 (HER2) protein which also stimulates growth of the cancer cells (Orrantia-Borunda et al., 2022). The cancer cells are acquired from the tumor and tested for ER, PR and HER2 to be assigned positive or negative for the particular type. Recently, additional markers are considered such as different micro RNAs and gene mutants (Orrantia-Borunda et al., 2022).

Primary treatment of breast cancer is surgery combined with chemotherapy, targeted therapy and/or radiation therapy; in surgery, a part or entire breast is removed, usually together with nearby lymph nodes to test whether the cancer has spread (Trayes & Cokenakes, 2021). Chemotherapy includes oral or intravenous drugs to kill the cancer cells. Radiation therapy is used to shrink tumor before surgery and after surgery to kill the possibly remaining cancer cells. Targeted therapy includes endocrine therapy to diminish the amount of hormones that could stimulate the growth of the cancer cells. Antibody drug conjugates (ADCs) contain monoclonal antibodies and cytotoxic substances to deliver these drugs to the targets: cancer cells with specific surface antigens. In this way the treatment is more effective and the risk of systemic toxicity is lower than in conventional chemotherapy. This method is particularly promising for HER2-low and triple (ER, PR and

HER2) negative breast cancer which previously lacked effective treatments. (Mark et al., 2023).

### 7. Effect of radiation on EVs of cancer cells and radiation induced bystander effect

About half of the cancer treatments involve radiotherapy (Huber et al., 2024). At the cellular level radiotherapy should cause DNA destruction in cancer cells (Jassi et al., 2024) by inducing changes in DNA repair and causing cell cycle arrest and apoptosis. As key mediators of cell communication, also EVs play an important role in these processes (Szatmári et al., 2019). EVs are selectively targeting cells and can modulate the effects of radiation (Ripoll-Viladomiu et al., 2024), promote immune and inflammatory responses (Huang et al., 2018) and transmit microRNAs (e.g. miR-603) to influence the response of cancer cells in glioblastomas (Ramakrishnan et al., 2020). Radiotherapy affects not only cancer cells but also surrounding normal tissue. The radiation-induced bystander effect describes the changes in non-irradiated cells induced by the signals from irradiated cells. Communication between the irradiated and non-irradiated cells via radiation-influenced EVs creates indirect radiation exposure which affects adaptation to radiation (Jassi et al., 2024).

### 8. Clinical studies on EVs in breast cancer diagnostics

Recent studies have focused on the lipid composition of plasma-derived EVs as diagnostic biomarkers for breast cancer. Using mass spectrometry-based lipidomics, researchers identified significant alterations in lipid classes, including phosphatidylcholines and sphingomyelins, in EVs from breast cancer patients compared to healthy controls. These distinct lipid profiles demonstrated high accuracy in differentiating cancer patients from non-cancer individuals, highlighting their potential as a non-invasive diagnostic tool for early detection and disease stratification (Nishida-Aoki et al., 2020). There are various molecular mechanisms by which EVs promote brain metastasis in breast cancer (Sakamoto et al., 2023): EVs deliver bioactive molecules, such as microRNAs and proteins, to recipient cells, thereby regulating signal transduction and protein expression levels; EVs from brain metastatic breast cancer cells disrupt the blood-brain barrier by altering tight junctions between endothelial cells and promoting tumor cell infiltration into the brain parenchyma; EVs influence astrocyte function, contributing to the establishment of a pre-metastatic niche that supports tumor growth (Sakamoto et al., 2023). Mizenko et al. (2024) analyzed 471 clinical trials performed from 2000 to 2022 to evaluate the status and challenges of EV-based diagnostics and therapies. They found that 70% of trials focus on diagnostics, with cancer (breast and lung) as the primary targets, while 18% explore therapeutic applications, particularly those using mesenchymal stromal cell-derived EVs for inflammatory, respiratory, and neurological disorders. Ultracentrifugation was the most common EV isolation method (31%), and RNA sequencing was the leading characterization tool (36%). However, only 36% of trials fully reported EV isolation/characterization protocols. Most trials were conducted in North America (42%) and Asia (36%), underscoring regional research dominance (Mizenko et al., 2024). The authors emphasize the need for standardized methodologies to address EV heterogeneity and enhance clinical translation. Zhang et al. (2023) assessed EVs in 80 patients with varying stages of the disease. The study demonstrated that the presence of specific EV markers correlated with tumor progression, offering a non-invasive approach for real-time monitoring (Zhang et al., 2023). Challenges such as standardizing isolation and characterization methods remain, with future research aiming to address these limitations to facilitate clinical translation.

### 9. Predictive value of EVs

Effects of EVs in cancer include angiogenesis, epithelial–mesenchymal transition, extracellular matrix remodelling, and immune escape (Tao and Guo, 2020). The predictive value of EVs lies in their ability to reflect real-time changes in the tumor microenvironment (Xu et al., 2024). Tumor-derived EVs contain a variety of biomolecules, including proteins, lipids, and nucleic acids, which reflect the molecular characteristics of the tumor,

providing valuable insights into disease progression and therapeutic response (Zhang et al., 2023; Xu et al., 2024). EVs are suggested to play crucial roles in intercellular communication, promoting tumor growth, metastasis, and drug resistance (Schwarzenbach & Gahan, 2020). They offer several advantages over traditional biomarkers, including their ability to capture tumor heterogeneity and their potential to track both primary tumors and metastatic sites (Lee et al., 2023; Vinik et al., 2020). The protein content of EVs can serve as a tool for monitoring therapeutic response and detecting early relapse in metastatic breast cancer (Tian et al., 2021; Zhou et al., 2021). Studies have identified specific EV proteins that correlate with breast cancer progression, recurrence, and drug resistance, suggesting their potential for personalized treatment strategies (Serrettiello et al., 2024; Tian et al., 2021). Recent advancements in proteomics and advanced analytical techniques, such as mass spectrometry, have enabled the identification of tumor-specific EV biomarkers, offering further insights into the molecular underpinnings of breast cancer (Bandu et al., 2024; Muttiah et al., 2024). The integration of EV analysis with other biomarkers, including circulating tumor DNA and small RNAs, enhances the sensitivity and specificity of early detection and monitoring of therapeutic efficacy (Koi et al., 2020; Rayamajhi et al., 2024).

## 10. Conclusions

EVs hold substantial promise as non-invasive biomarkers for breast cancer diagnosis, metastasis monitoring, and treatment response prediction. Their role in tumor biology, combined with advances in detection technologies, makes them a valuable tool for precision oncology and personalized cancer therapy. The potential use of EVs as delivery vehicles for targeted therapies opens new avenues for breast cancer treatment, with ongoing research exploring their role in immuno-oncology and drug delivery (Asleh et al., 2023; Wang et al., 2021).

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## Review

# The Problem of Modular Hip Endoprosthesis

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**Abstract:**

The mechanisms of premature fracture of modular-neck stems in two case studies: (I) when the neck and stem are both made of the same Ti6Al4V alloy, and (II) when the neck and stem are made from two different alloys, CoCrMo and Ti6Al4V alloy are presented. The study integrates two orthopedic patients who have undergone primary uncemented THA for usual indications in two orthopedic centers). Both centers are part of the national public health care system. Both surgeries were performed by two skilled orthopedic surgeons with more than 10 years of experience in THA. The survivorship of the modular neck of cast CoCrMo alloy was 24 months. The survivorship of the modular neck from Ti6Al4V alloy was 84 months. Advanced analyses were performed to assess the differences in the fretting, corrosion, and fatigue, using stereo light microscopy (SLM), scanning electron microscopy (SEM), X-ray energy-dispersive spectroscopy (EDS), and electron backscatter diffraction (EBSD). Patient demographic information, including sex, age, body mass index, survivorship of implants, and reason for the revision, was collected from medical records. The fretting and fatigue occurred on both modular stem retrievals, but the CoCrMo/Ti6Al4V alloy system suffered more corrosion due to additional galvanic corrosion and fractured earlier than the Ti6Al4V/Ti6Al4V metal alloy system. Both—metallic alloy systems used in this application are known to be highly corrosion-resistant, but the bio-tribo-corrosion processes need to be understood in detail and characterized so that appropriate improvements in design and materials can be made.

**Keywords:** Total hip arthroplasty; Modular neck; Ti6Al4V alloy; CoCrMo alloy; Corrosion

## 1. Introduction

Modular neck stems were introduced to hip endoprosthesis with the expected benefits of reducing pain and improving the range of motion and leg length (Azar et al., 2016; Baleani et al., 2023; Ellma & Levine, 2013). Besides classic stems, Wright Medical Technology/Microport, Stryker, Depuy, Lima Corporate, Zimmer, Adler Ortho, Cremasoli, and others are the recognized worldwide manufacturers of modular neck stems. The stems and the necks are available in different sizes and different neck angles, to tailor the implant to the individual patient. Increased implant modularity with modular necks made from Ti6Al4V and stems from the same alloy has generated interest in recent years because the various neck sizes, offsets, lengths, and design configurations allow the surgeon to optimize the range of motion and the patient's leg length. However, there have been concerns due to early *in vivo* fractures as well as adverse tissue reactions related to corrosion at the neck–stem interface (Baleani et al., 2023; Ellman & Levine, 2013; Wright et al., 2010; McTighe et al 2015; Park et al., 2018; Solarino et al., 2021; Maniscalco et al., 2020; Castagnini et al., 2023; Goffton et al., 2017; Fokter et al., 2017; ).

The presence of the neck–stem interface using titanium alloys makes the neck junction vulnerable to fretting, corrosion, and fatigue fracture. Several authors reported an early fracture of long modular necks made of Ti6Al4V alloy ( McTighe et al 2015; Park et al., 2018; Solarino et al., 2021; Maniscalco et al., 2020; Castagnini et al., 2023; Goffton et al., 2017; Fokter et al., 2017; Dolinar et al., 2018; Gorenšek & Jenko, 2018). Many of the femoral modular stems suffer from premature fractures after the total hip arthroplasty (THA) of the titanium alloy neck. Due to the results of *in vitro* investigations which presented better wear and mechanical properties, a cobalt–chromium–molybdenum neck was introduced (Castagnini et al., 2023; Goffton et al., 2017; Fokter et al., 2017; Dolinar et al., 2018; Jenko et al., 2018). CoCrMo neck fractures have also been reported after only a few months of implantation (Dolinar et al., 2018; Gorenšek & Jenko, 2018). Some reports have shown cases of fractured necks that could not be detracted from the stem pocket, requiring the replacement of the otherwise well-fixed femoral stems (Baleani et al., 2023; Wright et al., 2010; Solarino et al., 2021; Fokter et al., 2017; ).

The main aim of our work was to investigate mechanisms of premature failure of two case studies due to the fracture of the long neck in two different metallic alloy systems: the same alloy (neck and stem are both made from the same Ti6Al4V alloy with a survivorship of 84 months) and different alloys (neck made from CoCrMo alloy with better mechanical properties and a stem body made from Ti6Al4V alloy, with a survivorship of only 24 months) ( Dolinar et al., 2018; Jenko et al., 2018; Gorenšek & Jenko, 2018; Soteranos et al., 2013; Viceconti et al., 1997; Oladokun, et al., 2015; Gilbert et al., 1993). The research was a joint effort of orthopedic surgery clinicians and material scientists.

## 2. Methods

### 2.1. Stereo Light Microscopy (SLM)

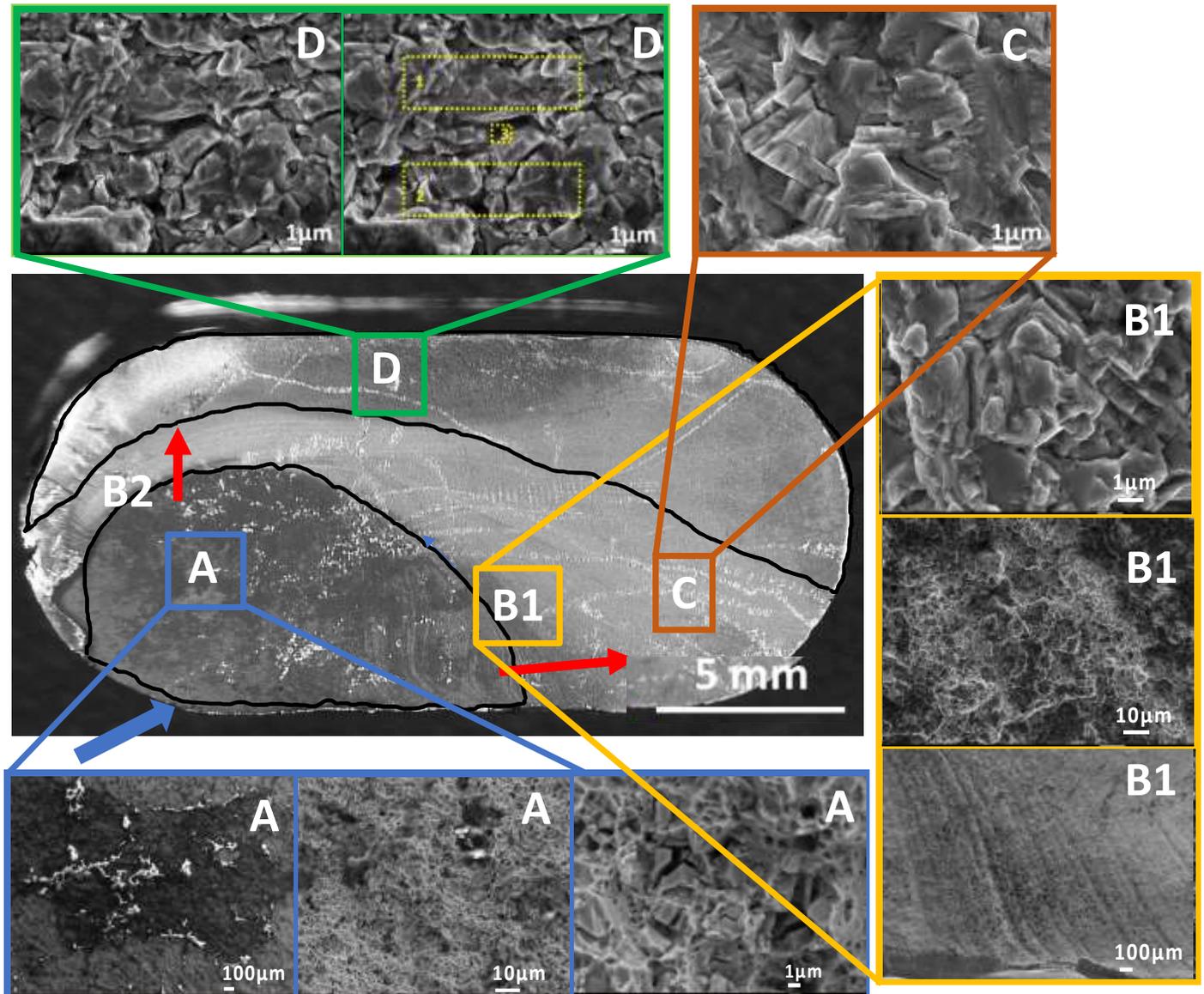
A stereo light microscope, Tagarno FHD trend, was used for the visualization of the fractured surfaces of the Ti6Al4V modular neck and the Co-Cr-Mo modular neck.

### 2.2. Scanning Electron Microscopy (SEM, SEM/EDS, SEM/EBSD) Analysis

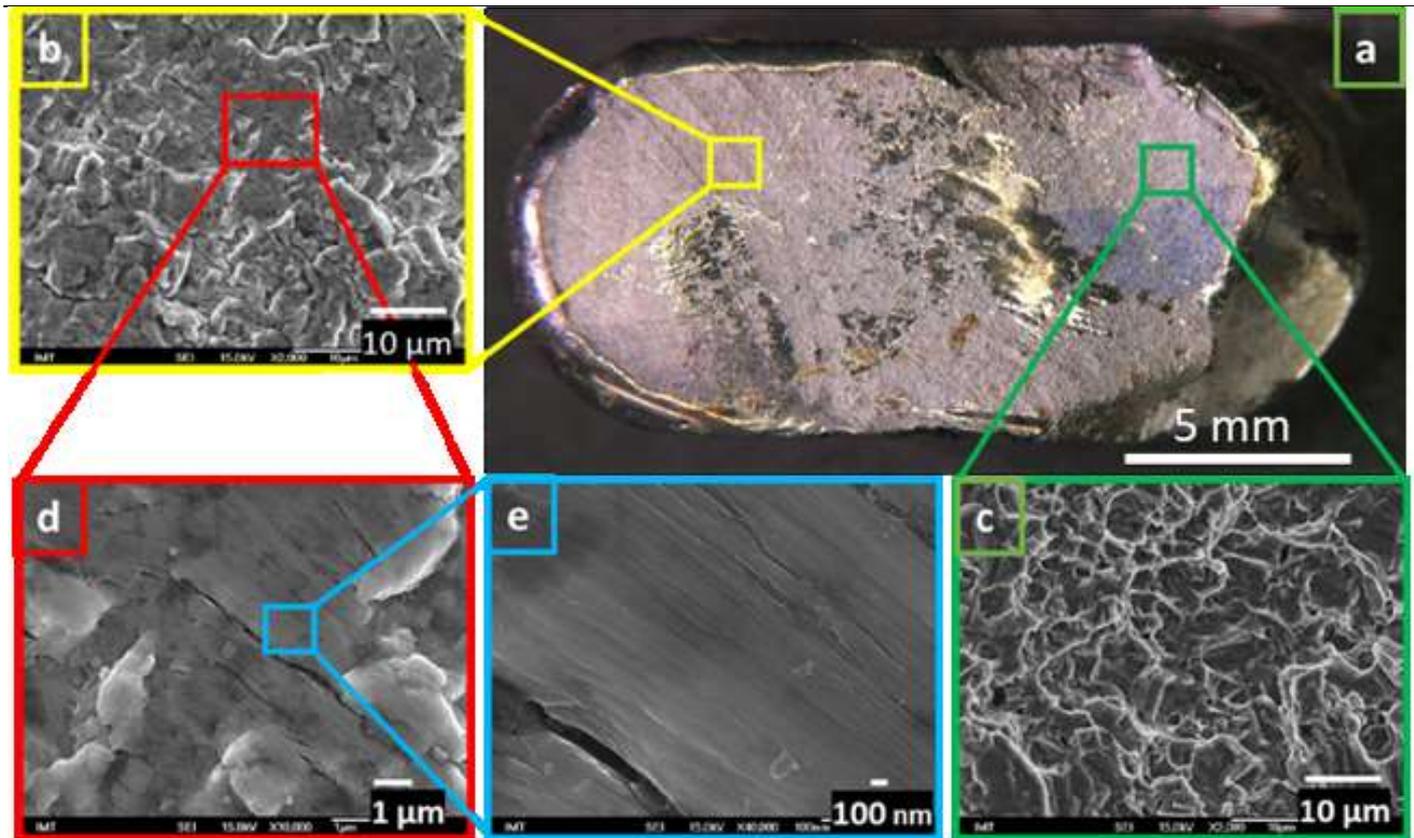
The morphology and microstructure of the fracture surfaces were analyzed using a scanning electron microscope, JEOL JSM 6500-F (JEOL Ltd., Japan). The SEM images were acquired using an accelerating voltage of 15 kV, with a current of about 500 pA and a working distance of 10 mm. Secondary electron and backscattered electron images were acquired. The elemental compositions of the samples were analyzed using Oxford INCA EDS analysis. The EDS spectra were acquired using a 15 kV and 1 nA beam, with an acquisition time of 60 s for each spectrum. The EDS spectra were analyzed using INCA Energy software to determine the elemental composition and distribution in the sample. Electron backscatter diffraction (EBSD) was also used to determine the type of carbides present in the microstructure. A Nordlys EBSD detector (HKL) and a Channel 5 data analysis suite were used. The EBSD patterns were acquired at 15 kV accelerating voltage and 2 nA current.

## 2. Results

Analysis of the surface and composition of the fractured surface of the two failed prostheses is shown in **Figures 1 and 2**. **Figure 1** presents the modular neck stem made of CoCrMo (neck) and Ti6Al4V (stem) alloys and **Figure 2** presents the modular neck stem made of the same alloy: Ti6Al4V (neck) and Ti6Al4V.



**Figure 1:** SLM of the fractured surface of CoCrMo neck on the main inside image represents the mechanism of CoCrMo alloy modular neck fracture. The blue arrow indicates the crack initiation; Zone-A the corroded fractured surface with deposited biological material is shown in three SE images in three blue marked panels: the left panel A at lower magnification, the detail of fatigue fracture and cracked carbides at higher magnification, panel A in the middle, and cracked carbide at highest magnification in the right panel A. The red arrows show the direction of fatigue crack propagation in the location B1 and location B2. The fatigue striations were observed in SE images in areas marked in yellow, the microstructures details are shown in middle and upper panels. At Zone C, the fracture surface is very rough and angular. The fracture surface shows several secondary cracks that are perfectly straight and short. Next to these flat cracks, larger secondary cracks can be seen, which are branched and longer than the flat ones. The lower SEI shows the crack propagation, and the middle and upper SEI shows the details at higher magnifications. The SE image of Zone-D marked green, shows a sudden fracture. Zone-D in the right panel shows areas of EDS analysis of the fracture surface (yellow squares marked in the right panel). From (Dolinar et al., 2023).



**Figure 2.** (a) Image of different fractured surface regions of fractured Ti6Al4V modular neck; (a–b) yellow-marked region and yellow-framed SE image of fatigue failure and with higher magnifications (b,d,e); (a,c) green-marked region and green-framed SE image with sudden ductile fracture. Organic deposits on fractured Ti6Al4V surface due to PE nanoparticle migration were found and visible as blue color (a). From (Dolinar et al., 2023).

### 3. Conclusions

The fretting, corrosion, and fatigue occurred on both neck–stem retrievals of the same (Ti6Al4V neck/Ti6Al4V stem) and different (CoCrMo neck/Ti6Al4V stem) metal systems. The cracked femoral neck made of CoCrMo alloy was dynamically loaded, exposed to a corrosive medium, and surrounded by a Ti6Al4V alloy with a different electrochemical potential. Due to the constant dynamic load and a combination of different materials in a corrosive medium, galvanic corrosion on the surface of the modular neck occurred. The different-metal system made of CoCrMo/Ti6Al4V suffered more corrosion than the same-metal system made of Ti6Al4V/Ti6Al4V alloy due to additional galvanic corrosion. The nature of the in vivo mechanisms causing the formation of the bio-tribo-corrosion processes needs to be understood and characterized so that appropriate changes in design and materials can be implemented.

**Conflicts of Interest:** The authors declare no conflict of interest.

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Reflection/Review

# Feline Tooth Resorption

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## Abstract:

Feline tooth resorption is one of the most common dental disorders in cats, characterized by a progressive loss of tooth substance. The aetiology of this disease is not completely understood. Multiple factors may play a role in etiopathogenesis of this disease, such as: age, diet, chronic oral inflammation, metabolic and endocrine diseases, anatomical abnormalities. Based on radiographic appearance, resorptive lesions are classified into three types. Several symptoms accompany this disease, including anorexia, dysphagia, halitosis and oral discomfort. Tooth resorption can be detected with a combination of visual inspection, examination under general anesthesia with a sharp dental explorer and intraoral dental radiography. Treatment of choice is extraction of the affected teeth, and crown amputation in teeth with type 2 lesions.

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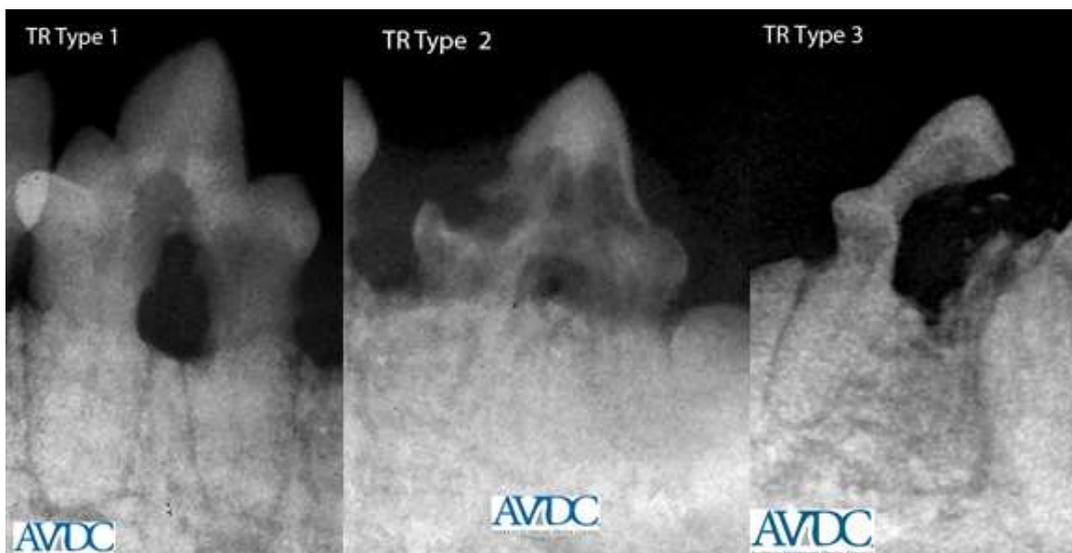
**Keywords:** Teeth; Cats; Resorptive lesions



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## 1. Introduction

Feline tooth resorption is one of the most common dental disorders in cats with a prevalence ranging from 30% to 60% (Ingram et al., 2001; Niemiec, 2012). This disease is characterized by a progressive loss of tooth substance (Girard et al., 2008). Mandibular third premolar teeth (307 and 407) are most commonly affected, but these lesions can occur in all types of teeth (Ingham et al., 2001; Gorrel, 2015). Age seems to be an important risk factor, along with diet and presence of other dental diseases. However, the aetiology of this disease is not understood completely (Niemiec, 2012; Pistor et al., 2023). What is known is that the resorption is caused by odontoclasts, cells responsible for the resorption of dentine and cementum. The resorption begins on the root surface, and then reaches the dentine (Niemiec, 2012). As the disease progresses, the pulp gets involved. Eventually, the enamel is affected and it either gets resorbed or it fractures off (Gorrel, 2015). Other factors that may play a role in etio-pathogenesis of this disease include: chronic oral inflammation, metabolic and endocrine diseases, systemic acidosis, local hypoxia, anatomical abnormalities, low urinary specific gravity, viral diseases (Reiter et al., 2005; Booij-Vrieling, 2009; Mestrinho et al., 2013). According to the American Veterinary Dental College (AVDC), there are three types of tooth resorption based on radiographic appearance. In type 1 lesions, focal or multifocal lucencies are present in the tooth, whereas the periodontal ligament space is normal and there is no bony replacement of the lost tooth structure. The radiopacity is also normal. In type 2 lesions, the periodontal ligament space is narrowed, radio-opacity is decreased and the lost tooth structure is replaced with bone. Type 3 represents a combination of types 1 and 2, with certain parts of a tooth showing type 1 lesions, and other parts showing type 2 lesions (Niemiec, 2012) (**Figure 1**).



**Figure 1.** Types 1, 2 and 3 of tooth resorption lesions (Source: Copyright AVDC®, used with permission).

### 1.1. Clinical presentation

Various symptoms accompany this disease, including anorexia, dysphagia, halitosis and oral discomfort (DuPont, 2005). Tooth defects are first noted at the gingival margin (Niemiec, 2012). Gingivitis is not always present, but when it is, it's recorded as mild, moderate and severe inflammation with redness, edema, bleeding and ulceration (Silness and Loe, 1964; Mestrinho et al., 2013). Many resorptive lesions are usually covered with gingival or granulation tissue which tend to bleed when touched (Debowes, 1994). Otherwise, these cases are often asymptomatic (DuPont, 2005). Type 1 lesions are usually associated with inflammation, whereas type 2 lesions create local gingivitis (Niemiec, 2012). In order to detect any of these lesions, a thorough oral examination under general anesthesia must be performed. Dental explorers are used to detect any irregularities at the surface of the tooth (Harvey et al., 2004) (**Figure 2**).



**Figure 2.** Clinical examination of right mandibular molar (tooth 409) using a dental explorer.

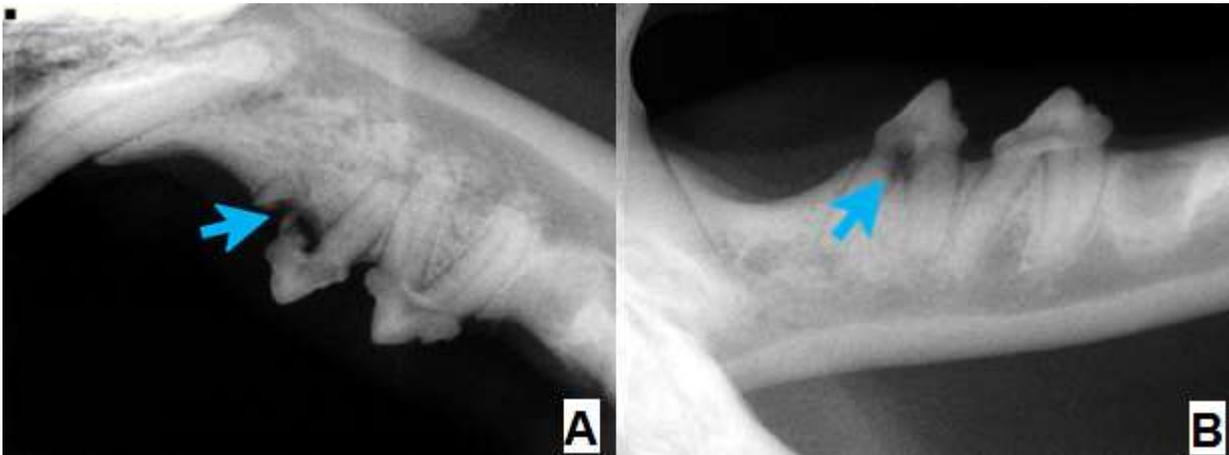
### 1.2. *Diagnosis and treatment*

Tooth resorption can be detected with a combination of visual inspection, examination under general anaesthesia with a sharp dental explorer and intraoral dental radiography. Visual inspection during an oral examination is the first step in diagnosing tooth resorption. These lesions can appear as tissue filled defects, along with inflamed associated gingiva (Niemic, 2012). However, this is not always the case, as the tissue may appear normal in early stages of the disease (Reiter et al., 2005). A dental explorer is used by placing it on the tooth and running it across the tooth surface, searching for rough areas. All tooth surfaces must be examined (Niemic, 2012). Dental radiography is essential in detecting tooth resorption lesions which are located below the gingival attachment, as well as differentiating between the types (Reiter et al., 2019). Full-mouth intraoral radiographs are especially recommended in cats 6 years of age or older (Niemic, 2009).

Various methods of treatment have been used historically. Some of them include: topical fluoride treatment (Lyon, 1990), restorations with glass ionomer or composite (Roes, 1996; Schweighart - Banzhaf and Benz, 1997), and use of alendronate which slowed the progression of tooth resorption (Mohn et al., 2009). Nowadays, the preferred treatment of choice is extraction. Considering that teeth with resorptive lesions are weak and dentoalveolar ankylosis is present, a surgical approach is recommended. In type 2 lesions, crown amputation is an acceptable treatment option, considering that it causes less trauma and faster healing (DuPont, 1995; Niemic, 2012).

## 2. **Experiences from the Clinic for Veterinary Dentistry at University of Sarajevo**

Since 2018, 41 cats with tooth resorption have been admitted to the Clinic for Veterinary Dentistry at University of Sarajevo – Veterinary Faculty. Admitted patients were of various breeds, predominantly domestic shorthair cats, aged 2 to 10 years old. 26 of them were males, and 15 females. All of the cats showed symptoms of halitosis and oral pain and discomfort. Prior to every detailed oral examination, anesthetic induction is performed with intramuscular medetomidine-hydrochloride (80 µg/kg), butorphanol (0.4 mg/kg) and ketamine (5 mg/kg). One perioperative injection of meloxicam (0.2 mg/kg SC) is administered. A complete periodontal probing of each tooth is performed, along with exploration of the tooth surface. Full mouth radiographs are obtained with the Schick intraoral x-ray system (SDX) (**Figure 3**).



**Figure 3.** A – Radiograph of the right side of the mandible in a 4-year-old mixed breed cat with a history of gingivitis, halitosis and anorexia. Blue arrow is showing resorptive lesions in tooth 407. B – Radiograph of the left side of the mandible in a 6-year-old mixed breed cat with chronic gingivitis (FIV positive). Blue arrow is showing resorptive lesions in tooth 307.

Depending on the type of lesions, treatment consists of extraction or crown amputation. Dental nerve blocks are performed using 2% lidocaine-hydrochloride (2 mg/kg). Postoperative instructions include the use of analgesics, along with semi-liquid food for the first couple of days after the procedure.

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Review

# Tracheal Injury after Endotracheal Intubation

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## Abstract:

Endotracheal intubation is a vital procedure in anaesthetized animals to deliver oxygen, volatile anaesthetics, and assist with ventilation. It also protects the airways from aspiration of gastric contents. However, improper intubation techniques or equipment can lead to complications, such as tracheal injury. Cats are particularly prone to tracheal rupture due to their sensitive tracheal anatomy and the overinflation of endotracheal tube cuffs. Dental procedures, which often require repositioning, account for most tracheal injuries in cats. Clinical signs, such as subcutaneous emphysema, coughing, and respiratory distress, may occur hours to days after the procedure. Diagnosis is based on history, clinical signs, radiographs, and tracheoscopy. While mild cases can be managed conservatively with oxygen supplementation and rest, severe injuries require surgical intervention. Proper tube selection, careful cuff inflation, and regular pressure monitoring are essential preventive measures to minimize the risk of tracheal injury. Early diagnosis and suitable management result in favourable outcomes for the affected animals.

**Keywords:** Endotracheal intubation; subcutaneous emphysema; dogs; cats; tracheal injury; tracheoscopy; dental procedures

## 1. Introduction

Endotracheal tubes are made of plastic, siliconized rubber, or red rubber and can be cuffed or uncuffed. Some tubes are reinforced with a metal spiral to prevent kinking or compression during extreme neck flexion. The size of the tube is determined by its internal diameter (in millimetres), while the external diameter determines its fit within the trachea.

The external diameter can vary depending on the material and whether the tube is cuffed. Selecting the correct tube size is critical (Dugdale et al., 2020). Endotracheal tubes with an internal diameter of 3.5 - 4.5 mm are appropriate for most cats (Hardie et al., 1999).

It is crucial to select the largest possible diameter that can still be used safely and allows for minimal inflation of the cuff. This is especially important for low-volume, high-pressure cuffs, as minimal inflation helps to seal the trachea while reducing the cuff pressure and thus minimizing the risk of damaging tracheal mucosa. A tube that is too small may increase breathing resistance or become obstructed with mucus, while a tube that is too large can cause tracheal damage.

Proper tube placement extends from the level of the incisors to the proximal third of the trachea, below the cricoid. The cuff should be inflated evenly on all sides to seal the space between the tube and the tracheal wall, reduce gas leakage into the atmosphere during mechanical ventilation, and prevent passage of contents from the pharynx into the distal trachea. It also helps to position the tube tip more centrally in the trachea, reducing the risk of tube tip obstruction and tracheal wall injury, which most commonly occurs at the tube tip (Dugdale et al., 2020) or cuff (Hardie et al., 1999; Dugdale et al., 2020).

Cats in particular have a sensitive dorsal tracheal ligament, which can rupture if the cuff is overinflated. This risk is increased during procedures that require repositioning, such as dental procedures or multiple x-rays. Most tracheal injuries in cats occur after dental procedures, accounting for 70% of cases (Hardie et al., 1999; Mitchell et al., 2000). Injuries have also been documented after bronchoalveolar lavage and excision of histiocytoma (Hardie et al., 1999), as well as after spaying, castration, oral surgery, and hip and stifle radiographs (Mitchell et al., 2000). More than half of the cats with a tracheal rupture were intubated using a high-volume, low-pressure cuffs (Bauer et al., 2009).

Unlike in cats, tracheal damage from endotracheal tube is very rare in dogs. Only two cases of tracheal stenosis have been reported – one occurring 9 days after surgery (Manabe et al., 2021), and another occurring 5 days after general anaesthesia for arthrodesis (Alderson et al., 2006). Additionally, an experimental study demonstrated tracheal necrosis caused by cuff pressure much higher than the recommended range (Su et al., 2017). Disconnecting the anaesthesia breathing system before repositioning the animal is essential to prevent tube rotation within the trachea, which could cause injury. Low-volume, high-pressure cuffs exert higher localized pressure on the tracheal mucosa, potentially causing necrosis if left in place for prolonged periods. Conversely, high-volume, low-pressure cuffs reduce localized pressure but may increase the risk of trauma during intubation or extubation (Dugdale et al., 2020). The longest tracheal ruptures were observed in cats intubated with high-volume, low-pressure tubes. No differences in occurrence were found between sexes or based on the age of the cats (Hardie et al., 1999).

Relaying solely on palpation of the pilot balloon alone makes it challenging to accurately assess the cuff pressure. However, cuff pressure can be measured with a manometer, especially in high-volume, low-pressure cuffs, where the pressure in the cuff approximately reflects the pressure on the tracheal wall. Ideally, during prolonged procedures, the cuff pressure should be regularly monitored, as the patient's muscle tone can change, as well as the compliance of the cuff as it warms up (Dugdale et al., 2020). Excessive pressure in the cuff can impair tracheal mucosal perfusion, potentially leading to ischemia or necrosis of the tracheal mucosa (Fudge, 2009; Su et al., 2017; Dugdale et al., 2020).

## 2. Causes of tracheal injury by endotracheal intubation

The potential causes of tracheal injury include:

- Overinflation of the cuff (Hardie et al., 1999; Fudge et al., 2009; Quandt, 2017; Dugdale et al., 2020).
- Incorrect tube placement (Hardie et al., 1999).
- Using an improperly sized tube (Hardie et al., 1999; Fudge et al., 2009).

- Failure to deflate the cuff before extubating (Hardie et al., 1999; Mitchell et al., 2000).
- Changing the position without disconnecting the tube from the anaesthetic breathing system (Dugdale et al., 2020; Mitchell et al., 2000).
- Intubation with the stylet (Mitchell et al., 2000).
- Chemical injury from inadequate rinsing of cleaning agents (e.g., glutaraldehyde, chlorhexidine, ethylene oxide (Alderson et al., 2006).

Tracheal injuries are more likely during dental procedures (Dugdale et al., 2020), where the cuff is often overinflated to prevent water aspiration into the lungs (Hardie et al., 1999; Quandt, 2017). It is possible that during a dental procedure, a tracheal rupture may occur due to changes in head position, which could cause excessive manipulation of the tube, leading to tracheal trauma (Mitchell et al., 2000).

Hardie et al. (1999) attempted to simulate a situation on cat cadavers where the endotracheal tube was not disconnected from the anaesthesia circuit, leading to tracheal twisting, but they were unable to induce a linear tear injury in the trachea.

### 3. Clinical signs

Clinical signs of tracheal rupture often develop some time after the procedure because the mediastinum, which closely surrounds the trachea, can initially prevent air from escaping. When coughing or irritation of the trachea occurs, air escapes into the mediastinum and subsequently into the subcutaneous tissues (Hardie et al., 1999).

Severe damage of trachea may result in subcutaneous emphysema or pneumothorax. Pneumothorax can occur if the pressure causes rupture of the mediastinum (Fudge, 2009). Clinical signs of tracheal rupture include subcutaneous emphysema in all affected cats (Figure 1), along with difficulty breathing, coughing, gagging, loss of appetite, and fever (Hardie et al., 1999; Mitchell et al., 2000). Other reported symptoms are vomiting, wheezing, drooling, pharyngeal swelling, submandibular oedema and dehydration (Mitchell et al., 2000).

The time between anaesthesia and the onset of clinical signs ranges from 4 hours to 7 days (Hardie et al., 1999). However, Mitchell et al. (2000) reported that cats were referred 5 hours to 12 days after the procedure, with a median time of 5.5 days. The most common cause of subcutaneous emphysema in cats that have recently undergone a procedure requiring endotracheal intubation is tracheal rupture (Bauer et al., 2009).



**Figure 1:** Severe subcutaneous emphysema in a cat 8 days after dental procedure.

#### 4. Diagnosis

Most cases are diagnosed based on the history and clinical signs (Bauer et al., 2009). Tracheal rupture is diagnosed using tracheal endoscopy, the preferred method, which reveals linear tears in the trachealis muscle. History along with clinical signs, such as subcutaneous emphysema, dyspnoea, and coughing guide initial diagnosis. Radiographs often reveal pneumomediastinum and subcutaneous emphysema (Hardie et al., 1999; Mitchell, 2000).

#### 3. Treatment

Both surgical and conservative treatments can be effective. The choice between conservative medical management and surgical intervention depends on the severity of the clinical signs and the clinician's judgment (Mitchell et al., 2000). Tracheoscopy can be used to determine the most appropriate treatment. Analgesia may be provided with a continuous infusion of ketamine,  $\alpha$ 2-adrenergic receptor agonists, or opioids, such as fentanyl. Alternatively, intermittent boluses can also be administered (Bauer et al., 2009).

##### 5.1. Conservative treatment

Conservative management is appropriate for cases with mild dyspnoea and subcutaneous emphysema, that do not worsen. Cats that respond to cage rest and oxygen supplementation should be treated conservatively. Subcutaneous emphysema is monitored through palpation.

Treatment includes:

- Cage rest.
- Oxygen supplementation (Mitchell et al., 2000).
- Sedation with butorphanol, midazolam, acepromazine, buprenorphine, or opioid-tranquilizer combinations should be used if needed (Quandt, 2017; Mitchell et al., 2000).
- Close monitoring for 12 to 72 hours (Mitchell et al., 2000).

##### 5.2. Surgical treatment

Surgical treatment is required for severe cases involving progressive dyspnoea and cyanosis, that do not improve with oxygen supplementation or in cases where subcutaneous emphysema worsens. Surgical repair is indicated for tears longer than 2 cm, with recovery requiring cage rest for 1 to 4 weeks (Bauer et al., 2009; Mitchell et al., 2000). During tracheoscopy, the animal cannot be intubated. Anaesthesia should be administered intravenously as a constant rate infusion, most commonly with propofol. The dose should be carefully titrated to effect to avoid apnoea. Supplemental oxygen should be provided throughout the procedure to prevent hypoxia. Total intravenous anaesthesia is also used for endoscopy, advanced imaging, and surgical repair of tracheal tears (Bauer et al., 2009).

If an animal with a tracheal rupture requires intubation for oxygenation and ventilation, the tube should be smaller than the lumen of the trachea and extend beyond the rupture. Positive pressure ventilation should be avoided or minimized (less than 10 cm H<sub>2</sub>O to prevent formation or worsening of subcutaneous emphysema and pneumothorax (Quandt, 2017). Subcutaneous emphysema usually resolves within 1 to 6 weeks, with a median of 2 weeks (Mitchell et al., 2000).

#### 4. Prognosis

All cats treated conservatively survived, whereas only two-thirds of those undergoing surgical treatment survived (Hardie et al., 1999). Cats that survived had no subsequent respiratory problems, either after surgical or conservative treatment (Mitchell et al., 2000).

#### 5. Preventive measures

To minimize the risk of tracheal injuries:

- Use the smallest effective cuff volume to seal the airway to prevent contamination of the operating room with anaesthetic gases and to ensure adequate ventilation of the cat.

- Gradually inflate the cuff in 0.5 mL increments, using a small syringe and checking for leaks to avoid overinflating the cuff, the cuff is properly sealed when gentle pressure on the reservoir bag no longer causes air leakage past the cuff of the tube.
- Disconnect the anaesthesia circuit before repositioning the animal.
- Monitor cuff pressure with a manometer during prolonged procedures (Hardie et al., 1999).

## 6. Conclusions

While essential for anaesthesia and airway management, endotracheal intubation carries a risk of tracheal injury if not performed carefully. Proper tube selection, careful cuff inflation, and vigilant monitoring during and after procedures are critical to minimizing complications. Early diagnosis and appropriate management, whether conservative or surgical, ensure a high likelihood of recovery without long-term consequences. In cats, tracheal ruptures most often occur after dental procedures, particularly at the cuff site, due to over-inflation of the cuff. These findings highlight the need for precise cuff management and cautious handling during procedures, especially those requiring repositioning, to prevent such injuries.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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Review

# Brachycephaly in Cats: A Silent Problem in Feline Health

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## Abstract:

Brachycephaly, morphological trait characterised by a shortened skull relative to its width, is a prominent feature of certain feline breeds, including Persians, Exotic Shorthairs, Himalayans, British Shorthairs, British Longhairs, and Scottish Folds. While selective breeding has favoured this trait due to human preferences for neotenous appearances, severe brachycephaly is associated with a wide range of health complications. These include neurological, respiratory, ocular, dental, gastrointestinal, reproductive, and dermatological disorders. This literature review explores the systemic impact of brachycephaly on feline health, highlighting the need for ethical breeding practices and improved veterinary care to mitigate its negative effects.

**Keywords:** Brachycephaly; Feline Health; Ethical Breeding; Skull Morphology; Cat Welfare

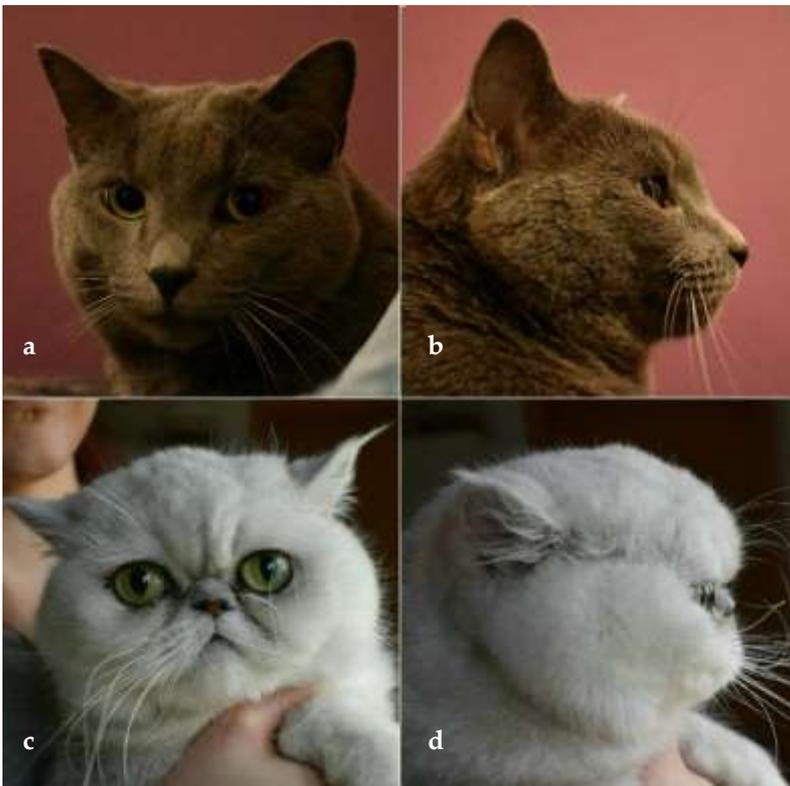
## 1. Background

Brachycephaly is an anatomical condition where the craniofacial conformation of the skull is significantly shortened (Gleason, et al., 2022), resulting in round face and large eyes (Berteselli et al., 2023). While this trait is often considered aesthetically desirable, it poses several health risks for affected cats. In recent decades, selective breeding has intensified the severity of brachycephaly in certain breeds, leading to an increase in associated medical conditions. Despite growing awareness, the long-term implications of these abnormalities remain insufficiently explored (Farnworth, 2016; Berteselli et al., 2023).

The aim of this review is to provide a comprehensive overview of the health challenges associated with brachycephaly and to emphasize the importance of ethical breeding and responsible ownership. This literature review explores how anatomical skull conformation affects the health of brachycephalic cat breeds, assessing the severity and systemic consequences of brachycephaly while highlighting the need for ethical breeding practices and improved veterinary care.

## 2. Brachycephaly

Brachycephaly is a term used to describe a morphological trait in which the length of a cat's skull is significantly reduced compared to its width (Mullen et al., 2004). Therefore, the shape of the brachycephalic cat's head appears to be rounder, face is flatter from the forehead to the snout. Eyes are bulging and do not follow the shape of the skull, while eye sockets are shallower (Sieslack et al., 2021). The nasal bridge is shortened to the extent that it is almost not visible. The lower jaw appears to be disproportionately pushed forward and upwards compared to the upper jaw (Schlueter et al., 2009; Berteselli et al., 2023; Morel et al., 2024) (**Figure 1**).



**Figure 1.** Comparison of the front (a) and side profile (b) in a non-brachycephalic cat, compared to a front (c) and side profile (d) in a brachycephalic cat (Photo: Jarnovič L).

It should, however, be noted, that the severity of brachycephaly in cats varies, and can be categorized into four distinct grades (Schlueter et al., 2009; Bessant et al., 2018; Anagrius et al., 2021) (**Table 1**). For classification, the cephalic index, a ratio measuring skull width relative to length, is used (Sieslack et al., 2021; Ziemann et al., 2023).

**Table 1.** Grading of the severity of brachycephaly in cats, with a description of each degree (Schlueter et al., 2009; Bessant et al., 2018; Anagrius et al., 2021).

Degree of Brachycephaly	Description
Mild	Subtle facial flattening with slight shortening of the skull. The nasal bridge remains moderately defined, and eyes are normal. Health issues are minimal.
Moderate	More noticeable facial flattening and narrower nostrils. Eyes may be slightly prominent. Respiratory problems such as snoring and exercise intolerance along with mild ocular and dental issues, are common.
Severe	Significant facial flattening, compressed nose, and prominent eyes. Respiratory distress, exercise intolerance, and pronounced dental malocclusion are common. Eye issues like corneal ulcers may occur.
Extreme	Severely flattened face, pushed-in nose, and bulging eyes. Severe breathing and difficulties, significant dental and neurological problems, and frequent ocular injuries are present.

### 2.1. *Brachycephalic Cat Breeds*

The condition is particularly prevalent in breeds like the Persian, Exotic Shorthair (Schlueter et al., 2009; Farnworth et al., 2016; Bessant et al., 2018; Plitman et al., 2019; Anagrius et al., 2021; Berteselli et al., 2023), Himalayan, British Shorthair, British Longhair, and Scottish Fold. These cats have been bred to emphasize features that align with human psychological preferences for “baby-like” appearances, such as large eyes and small noses (Schlueter et al., 2009; Bessant et al., 2018; Plitman et al., 2019; Berteselli et al., 2023).

## 3. Impact of brachycephaly on health

Cats with extreme skull shortening exhibit a significantly compressed facial structure, often leading to respiratory difficulties. Other complications linked to brachycephaly include neurological, ocular, dental, gastrointestinal, behavioural, reproductive and dermatological disorders (Schlueter et al., 2009; Bessant et al., 2018; Plitman et al., 2019; Erjavec et al., 2020; Anagrius et al., 2021).

### 3.1. *Neurological Issues*

Cats with severe brachycephaly often experience structural deformities within the skull (Huizing et al., 2017). One notable condition is cerebellar herniation, where the cerebellum is displaced through the foramen magnum due to reduced cranial space (Huizing et al., 2017; Bessant et al., 2018; Erjavec et al., 2020; Sieslack et al., 2021). This leads to motor dysfunction, ataxia (Erjavec et al., 2020; Anagrius et al., 2021), and, in some cases, hydrocephalus that intensifies pressure on neural structures (Huizing et al., 2017). Such cats may suffer seizures, disorientation and blindness (Erjavec et al., 2020).

### 3.2. *Respiratory Complications*

Respiratory difficulties are a hallmark of brachycephaly (Bessant et al., 2018; Brunner et al., 2023). Some cats exhibit Brachycephalic Obstructive Airway Syndrome, or better known as BOAS (Farnworth et al., 2016; Anagrius et al., 2021; Berteselli et al., 2023), a combination of congenital defects like stenotic nares, elongated soft palates (Mullen, 2004), and compressed nasal turbinates (Farnworth et al., 2016; Erjavec et al., 2020; Sieslack et al., 2021). These abnormalities obstruct airflow, resulting in labored breathing, snoring, and exercise intolerance (Mullen et al., 2004; Farnworth et al., 2016; Anagrius et al., 2021; Berteselli et al., 2023; Gleason et al., 2023). Severe cases can escalate to laryngeal collapse (Mullen et al., 2004; Farnworth et al., 2016; Anagrius et al., 2021; Brunner et al., 2023) that might require, though not always successful, surgical intervention (Brunner et al., 2023).

### 3.3. *Sleep Disturbances*

Cats with severe BOAS frequently experience sleep apnoea (Mullen et al., 2004; Berteselli et al., 2023), snoring (Farnworth et al., 2016; Anagrius et al., 2021; Berteselli et al., 2023) and restless sleep due to difficulty maintaining clear airways (Berteselli et al., 2023).

### 3.4. *Ocular Disorders*

The flattened skull structure often compresses or displaces the nasolacrimal ducts, leading to tear overflow, or epiphora (Schlueter et al., 2009; Erjavec et al., 2020; Anagrius et al., 2021; Sieslack et al., 2021; Berteselli et al., 2023). Namely, in severe brachycephalic cats, the proximity between the roots of the canine teeth and the nasolacrimal duct is significantly reduced, with the roots sometimes directly adjoining the duct. Consequently, the duct is forced to course beneath the canine teeth, forming a V-shaped trajectory before entering the nasal cavity (Schlueter et al., 2009). Protruding eyes are particularly vulnerable to corneal ulcers and entropion—a condition where the eyelids roll inward, irritating the cornea (Anagrius et al., 2021; Sieslack et al., 2021).

### 3.5. *Oral and Dental Health*

Dental malocclusion is another major issue (Ziemann et al., 2023), where the dorsorotation of the jaws causes overcrowding of teeth (Schlueter et al., 2009; Erjavec et al., 2020; Sieslack et al., 2021). This increases the risk of periodontal disease, gingival injury, and painful abscesses (Erjavec et al., 2020; Gleason et al., 2023). Traumatic occlusion, where teeth impinge on soft tissues, can lead to chronic inflammation and even pyogranulomas (Ziemann et al., 2023). These conditions make eating difficult, compromising nutrition and digestion (Gleason et al., 2023).

### 3.6. *Gastrointestinal Problems*

Respiratory stress often forces brachycephalic cats to breathe through their mouths, leading to aerophagia. This can result in regurgitation (Anagrius et al., 2021; Berteselli et al., 2023) gastroesophageal reflux, and poor digestion. Dental malocclusion further exacerbates eating difficulties, causing hypersalivation and messy eating behaviours (Gleason et al., 2023).

### 3.7. *Reproductive Challenges*

Queens with extreme brachycephaly often face dystocia (Bessant et al., 2018; Anagrius et al., 2021; Berteselli et al., 2023) or difficult labour, due to their narrow pelvic structure (Erjavec et al., 2020) and the disproportionately large head size of brachycephalic breeds (Morel et al., 2024). Caesarean sections are often required (Plitman et al., 2019), further highlighting the physical strain these traits impose.

### 3.8. *Dermatological Concerns*

Deep skin folds around the face create an environment prone to bacterial and fungal infections. Without meticulous grooming, these cats may develop painful skin conditions (Sieslack et al., 2021). Persians and Himalayans, with their dense coats, require regular grooming to avoid matting (Berteselli et al., 2023).

## 4. **Potential causes of health concerns being overlooked**

Studies indicate that the prevalence of health complications in brachycephalic cats varies by breed and severity of skull compression. Despite these findings, many prospective pet owners remain unaware of the medical challenges associated with extreme brachycephaly. Veterinarians are significantly more aware of brachycephalic dog health issues than owners and breeders (Åsbjær et al., 2024). While brachycephalic dogs often display obvious signs of breathing difficulties, in brachycephalic cats, their subtle face expressions make it difficult to recognize discomfort or respiratory distress (Berteselli et al., 2023). Unlike dogs, cats are generally less active, do not go for walks in hot weather, and tend to rest more

throughout the day. As a result, clinical signs of respiratory distress may be less pronounced or go unnoticed for longer periods. This lack of outward symptoms likely leads to an even greater underestimation of their health challenges than in dogs, making it a big silent threat to feline well-being.

## 5. Ethical and welfare considerations

Preventing the worsening of brachycephalic-associated health issues requires collaboration among breeders, veterinarians, cat owners and others (Åsbjer et al., 2024). Breeders should prioritize moderate facial features over extreme ones. By selecting for healthier traits, they can reduce the incidence of severe brachycephaly. Incorporating genetic screening into breeding practices can help eliminate deleterious traits and promote a more sustainable feline population (Mills, 2019; Sieslack et al., 2021; Morel et al., 2024).

### 5.1. The role of breeders

Breeders play a critical role in mitigating the negative effects of brachycephaly by prioritizing well-proportioned facial structures over extreme conformation. However, breeding conditions are often influenced by beauty standards set in competitions, making judges crucial in shaping breeding trends (Plitman et al., 2019). Their influence can encourage breeders to prioritize animal welfare by promoting traits that support long-term health and vitality. Genetic screening, and responsible selection of breeding pairs further help reduce the incidence of severe abnormalities (Morel et al., 2024).

### 5.2. Veterinary care and management

Veterinarians play a critical role in educating, diagnosing conditions and managing health problems early. Regular check-ups and preventive care are essential for detecting potential issues before they become severe. Advanced imaging techniques such as MRI or CT scans can help identify anatomical abnormalities (Huizing et al., 2017), allowing for more precise treatment planning. Additionally, specialized respiratory assessments and dental evaluations are crucial for brachycephalic cats, as they are prone to airway obstruction and malocclusions.

In severe cases, surgical interventions—though not always common—may significantly improve quality of life. Procedures such as airway correction surgery or nostril widening can enhance breathing capacity, while dental extractions may alleviate discomfort caused by overcrowding. A multidisciplinary approach, involving veterinary specialists in surgery, dentistry, and neurology, can further optimize long-term care and well-being (Morel et al., 2017).

### 5.3. Owner awareness and responsibility

Cat owners must be educated about the potential health risks of brachycephalic breeds prior to deciding of having one (Plitman et al., 2019). Routine veterinary check-ups, weight management, and creating stress-free environments are crucial. Owners should also be encouraged to support ethical breeding practices and avoid promoting extreme traits (Mills, 2019; Morel et al., 2024).

## 6. Conclusion

Brachycephaly in cats presents a significant yet often overlooked welfare concern. Affected individuals frequently experience chronic respiratory compromise due to stenotic nares, elongated soft palates, and compressed nasal turbinates, leading to Brachycephalic Obstructive Airway Syndrome (BOAS). Ocular pathologies, including corneal ulceration, entropion, and epiphora, are prevalent due to the altered orbital conformation. Severe dental malocclusions result in traumatic occlusion, periodontal disease, and feeding difficulties, compromising overall health and nutrition. Neurological abnormalities such as cerebellar herniation and hydrocephalus contribute to ataxia, seizures, and visual impairment. Unlike brachycephalic dogs, cats often mask clinical distress, leading to underdiagnosis and delayed intervention. Addressing this issue requires a multidisciplinary approach, where veterinarians must advocate for early detection and intervention, breeders must prioritize functional conformation over exaggerated traits, and owners must be informed of the in-

herent health risks. If left unaddressed, the health challenges associated with brachycephaly will persist unnoticed, reinforcing its status as a silent yet profound threat to feline well-being.

**Conflicts of Interest:** The authors declare no conflict of interest.

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Research

# Effective Teaching and Learning Opportunities During Clinical Education from the Students' Perspective - Prospective Cross-sectional Study

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## Abstract:

The study aimed to explore physiotherapy students' perceptions of effective teaching and learning opportunities (TLOs) and the key attributes of clinical teachers during clinical education. Understanding these perceptions can help improve clinical teaching practices and enhance learning experiences. A prospective cross-sectional study was conducted among final-year physiotherapy students at the Faculty of Health Sciences, University of Ljubljana, Slovenia, during the 2021/2022 academic year. An adapted self-administered, structured questionnaire was used to collect data. Descriptive statistics were applied to analyze students' preferences regarding TLOs and clinical teacher attributes. A total of 46 students (61% response rate) participated. The most valued TLOs included patient management demonstrations (93%), feedback sessions (85%), and discussions with clinical teachers (76%). The most important clinical teacher roles were identified as feedback provider (93%), knowledge provider (91%), and mentor (89%). Key attributes of effective clinical teachers included good communication (89%), respect for students (78%), and approachability (72%). Students perceive patient management demonstrations, feedback, and discussions as the most beneficial TLOs. Effective clinical teaching is associated with clear communication, respect, and accessibility. The findings highlight the need for structured guidelines to support clinical educators and ensure consistency in physiotherapy education.

**Keywords:** teaching and learning opportunities; clinical teacher; attributes; physiotherapy; clinical education



## 1. Introduction

Clinical education offers a unique learning experience as opposed to classroom teaching. The student works with a clinician to translate the theoretical knowledge acquired in previous years into professional skills and behaviours (Rindfleisch et al., 2009). On the other hand, clinical education provides a less controlled learning environment than classroom instruction, which sometimes makes it difficult for clinical educators to plan a stable and equitable clinical education experience for all students (Ernstzen et al., 2009). In the 'real world', the objectives of the healthcare institution in which teaching takes place are centred on the provision of quality healthcare to patients. In this context, the physiotherapy student's education may take a secondary role to patient care. Clinical education therefore becomes less of a structured approach to learning and more of a series of learning opportunities for the student to take up when they can.

In higher education, the rise of student-centred learning has led to a shift in emphasis from traditional 'teaching' to 'learning' (O'Neill & McMahon, 2005). In this model, the teacher serves as a facilitator rather than just a transmitter of knowledge, while students take a more active role in their education. This approach transfers the power from the teacher to the student (Milanese et al., 2013). A student-centred approach is particularly well suited to clinical education, where the unpredictability of the learning experience can disadvantage passive students. Actively engaged students, on the other hand, are more likely to take advantage of and benefit from the learning opportunities presented to them (Ernstzen & Bitzer, 2012).

Considering the problems mentioned above (e.g. lack of control over the setting, transition to student-centred learning), understanding the experience from the student's perspective can help identify strategies to improve the learning process and promote engagement (DeClute & Ladyshefsky, 1993; Hobbs et al., 2000; Crosbie et al., 2002).

In clinical education, the learning environment is influenced by the teaching and learning opportunities offered, the roles and attributes/characteristics/personality of the clinical teacher, the student and the patient, the models of clinical education used, the assessment of students and the atmosphere and facilities of the healthcare institution (Ernstzen & Bitzer 2006; Ernstzen & Bitzer, 2012). Two aspects of the learning environment, namely the teaching and learning opportunities (TLOs) and the roles and attributes of clinical teachers, have been shown to be the most significant factors influencing learning (Chan, 2001).

Clinical education TLOs are learning activities that the student encounters in the clinical environment that allow the student to actively build and integrate their theoretical and practical knowledge, through the experience of clinical practice (Ernstzen et al., 2009). Little is known about teaching-learning strategies that clinical teachers use in clinical education and the perceived efficacy of these (Kilminster et al. 2007). Knowledge of these aspects may lead to appropriate changes made in clinical education thereby enhancing learning in the clinical environment. As teaching supports learning, it is useful to determine which teaching-learning activities students view as effective and useful.

Although the importance of clinical education is eagerly emphasised, recommendations for optimal TLOs are inconsistent (Van der Hem-Stokroos, 2005). There is currently no standardised guide to help clinical teachers in physiotherapy organise their teaching method (Robertson et al., 2003). In other words, each clinical teacher teaches differently as it depends on their own judgement. This leads to different support and teaching by clinical teachers and different learning for individual students and in the same clinical rotation (Hobbs et al., 2000; Milanese et al., 2013). These voices are of concern in clinical education as they can lead to inconsistency and inequity in student learning experiences, lack of validity and reliability in assessment, varying standards in clinical teaching, varying levels of motivation among clinical teachers, and a lack of time and staff for clinical education (Delany & Bragge, 2009; Hartley et al., 2019).

Previous researchers found that the attributes of the clinical teacher and the relationship between the student and the teacher have a powerful effect on learning (Kilminster & Jolly, 2000). Attributes such as approachability, enthusiasm, communication skills and the desire to share knowledge have been identified as important attributes for clinical teachers (Bennett, 2003; Ramakrishnan & Bairapareddy, 2020). Behaviours such as being a competent physiotherapist, being available, answering questions without belittling the

student, and providing useful and constructive feedback were perceived by students as the most effective clinical teaching behaviours (Onuoha, 1994; Ernstzen & Bitzer, 2012). The aim of the study was to find out what final year physiotherapy students enrolled at the Faculty of Health Sciences at the University of Ljubljana in Slovenia (FHSUL) consider to be effective teaching and learning opportunities during clinical education and what they consider to be the role and attributes of an effective clinical teacher.

## 2. Material and Methods

The study follows a prospective cross-sectional design among the cohort of final year physiotherapy students enrolled for the 2021/2022 academic year at the FHSUL in Slovenia. FHSUL offers a 3-year undergraduate program in physiotherapy which consists of theoretical studies (46%) and practical training at the university and in clinical placement (54%). Students must complete a 20-week of minimal 700 hours clinical education throughout the curriculum.

A self-administered, structured questionnaire was used for data collection based on questionnaire used by Milanese et al. (2013) and Ernstzen et al. (2009) in studies of physiotherapy students' perceptions on effective practices for teaching and learning in the clinical setting. Before we translated the questionnaire into Slovenian, we received permission from the author Ernstzen et al. (2009) of the original questionnaire to use the questionnaire and adapt it to our context. The translation process of the original questionnaire from English into Slovenian followed the recommendations of Squires et al. (2013), which consider cross-cultural aspects of translation, where questions, expressions and concepts from the source culture (English) are carefully adapted to the target culture (Slovenian), considering the specific characteristics of the Slovenian language, cultural norms, values and context. Ten participants assessed the suitability of the questionnaire's content in pilot study and evaluated its key features, including clarity, simplicity, conciseness and time required to complete the questionnaire, on a scale of 1 (strongly disagree) to 5 (strongly agree). The average rating for clarity was 4.6 ( $\pm 0.5$ ), for simplicity 4.4 ( $\pm 0.7$ ), for conciseness 4.6 ( $\pm 0.52$ ) and for time suitability 3.8 ( $\pm 0.63$ ).

The questionnaire asked for basic demographic information (age, gender) and specific questions regarding their clinical placements. From the fifteen TLOs listed in the questionnaire, students selected the five most important ones where they learnt the most. Of the ten roles that are most important for the clinical teacher and the twelve attributes of the clinical teacher that the students consider necessary for optimal learning and that were listed in the questionnaire, the students selected the five most important.

An electronic version of the questionnaire was sent to final year students via the website MojaAnketa.si.web. Before completing the questionnaire, all participants were informed of the following: the purpose of the survey, that participation was voluntary and that the answers would remain anonymous. Completion and submission of the questionnaire implied informed consent.

Collected data were entered into Microsoft Excel spreadsheets, and analysis was performed in both Excel and IBM® SPSS Statistics (v22) (Armonk NY, US). Descriptive statistics, including frequencies and percentages for categorical variables, and mean and standard deviation (SD) for continuous variables were calculated to describe the demographic data.

## 3. Results

Of the total number ( $n = 76$ ) of students enrolled in the final year of the 2021/2022 academic year, 46 students completed the questionnaire in full during the study period (response rate of 61%). The participants included 36 female and 10 male students with an average age of 22.7 years (range 22–29 years).

The perceived learning values of the different TLOs are shown in **Table 1**. The five most important useful domains for providing TLOs to students was demonstration of patient management, followed by feedback and discussion with the clinical teacher, clinical tasks and one-to-one learning sessions.



**Table 1.** Students' views on teaching and learning opportunities/activities during which students learn the most.

Teaching and learning opportunities	Percentages and (number) of students who selected the activity
Demonstrations of patient management	93% (43)
Feedback	85% (39)
Discussions with the clinical teacher	76% (35)
Clinical tasks	41% (19)
One-to-one learning sessions	37% (17)
Reflection	28% (13)
Discussion with peers	26% (12)
Group-learning sessions	22% (10)
Learning on own	22% (10)
Mock assessment	17% (8)
Coaching	17% (8)
Questioning	13% (6)
Peer assessment	9% (4)
Lectures	7% (3)
When learning is facilitated	4% (2)
Other	2% (1)

The participants were asked to choose what they considered to be the most important roles of the clinical teacher. The results are shown in **Table 2**. From the results of the questionnaire, the five most important roles of the clinical teacher were selected: reflector/feedback provider, knowledge provider, mentor and demonstrator of techniques.

**Table 2.** Clinical teacher role

Clinical teacher role	Percentages and (number) of students who selected the role
Reflector/feed-back provider	93% (43)
Knowledge provider	91% (43)
Mentor	89% (41)
Technique demonstrator	80% (37)
Counsellor	70% (32)
Role model	35% (16)
Assessor/Evaluator	15% (7)
Facilitator of learning	13% (6)
Questioner	9% (4)
Friend	4% (2)
Other	0% (0)

Students identified the importance of the communication skills of the clinical teacher as the most important attribute. Other important attributes of clinical teacher attributes were respecting the students and approachability. The attributes that received the lowest rating in this study were that the clinical instructor is enthusiastic, a listener, and self-confident (**Table 3**).



**Table 3.** Clinical teacher attributes

Clinical teacher attributes	Percentages and (number) of students who selected the attribute
Good Communication	89% (41)
Respects the student	78% (36)
Approachability	72% (33)
Shows concern for student	61% (28)
Supports the student	59% (27)
Organised	37% (17)
Not prejudiced	35% (16)
Gives recognition for student abilities	33% (15)
Interpersonal skills	17% (8)
Enthusiasm	11% (5)
Listener	4% (2)
Self-confident	4% (2)
Other	0% (0)

#### 4. Discussion

The objective of this study was to explore physiotherapy students' perceptions of the teaching and learning activities associated with clinical education and how they perceive the key roles and attributes of the clinical teacher. Our students valued demonstrations of patient management by a clinical teacher as one of the most valuable clinical TLO. Demonstrations of patient management by the clinical teacher are usually situations where he/she performs the patient management step by step, demonstrating the subdivisions of the patient management. These subdivisions are going through the patient folder, performing the interview, planning and performing the physical assessment, as well as management strategies for the patient. A demonstration on patient management may help the student to determine where to start the process of patient management, what to assess, and promote decision-making skills (Stoikov et al., 2022). Observing the teacher performing the demonstration may be seen as learning by observation, and performing the demonstration may be seen as learning by doing (Torre et al., 2006). On the other hand, teacher-led demonstrations may be seen more than as passive learning by the student (Kilminster et al., 2007).

Another important TLO in this study was feedback. Van der Hem-Stokroos et al. (2005), Allen & Molloy, (2017) and Wjibenga et al. (2019) point out that feedback should be a key element in clinical education programmes. Feedback to students should be clear and unambiguous so that they are aware of their mistakes and weaknesses (Kilminster et al., 2007). Our results support the findings of Kilminster & Jolly (2000), who emphasise the importance of feedback for students' self-confidence and for a good relationship between students and teachers.

Discussions with the teacher were selected by most students as another valuable TLO in our study. Discussions about a specific patient appear to be useful in facilitating learning because they actively engage the student, as through discussions students practise thinking through problems, organising concepts, formulating arguments and counterarguments, evaluating the evidence for their own and others' positions, and responding thoughtfully and critically to different points of view (Milanese et al., 2013;

Stoikov et al., 2022). A discussion is therefore a collaborative learning event that offers the opportunity to gain knowledge and insights through the exchange of ideas and opinions. For our cohort of students, the use of TLOs that were least frequently selected in the questionnaire and were not directly related to a patient (mock assessment, coaching, questioning, peer assessment, lectures) needs to be reconsidered.

The data obtained from our study showed that students perceive the most important role of the clinical teacher as a reflector/feedback and knowledge provider, mentor and demonstrator of techniques. The role of the clinical teacher as a facilitator of learning was rated low by the students in our study. These findings are not in line with current professional evidence which postulates that the role of the clinical teacher is to facilitate learning opportunities and maximise the clinical experience within the duration of the placement (CSP Chartered Society Physiotherapy, 2020). However, the role of the teacher as both a provider of feedback and knowledge and a facilitator of self-responsibility for learning appears to be contradictory. Harden & Crosby (2000) may offer a viewpoint that could explain the students' perspective. They state that the teacher as a knowledge provider takes on a whole new meaning in the clinical context. Rather than providing knowledge by delivering it on to students (e.g. in the form of a lecture), in the clinical context the teacher passes on knowledge that relates to the specific condition of the patient. This situation creates a strong context that facilitates learning due to the immediate relevance of this knowledge.

The attributes of clinical teachers that played an important role for our students were identified as good communication, respect for students, approachability, interest in students, and support for students. Communication skills of the clinical teacher was the most important attribute from the students' perspective. It has been documented that physiotherapy students have had negative experiences on placement where they have experienced problems with communication with the clinical teacher (Ramakrishnan and Bairapareddy, 2020), an unapproachable clinical teacher (Meyer et al., 2017) and a lack of mutual appreciation of the roles of teacher and student (Delany & Bragge, 2009). The relationship between students and the clinical teacher has been shown to be a better predictor of client outcome than the ability or helpfulness of the clinical teacher (Kilminster & Jolly, 2000).

#### 4.1. Recommendations

As the main aim of our study was to determine which of the TLOs were considered by the physiotherapy students to be effective in enhancing learning in the clinical setting, these activities - demonstration of patient management, feedback and discussion with the clinical teacher - should therefore form the basis of the clinical teaching programme. At the FHSUL in Slovenia, there is currently no standardised guide to help physiotherapy clinical teachers organise their teaching method, as it is not compulsory for physiotherapists working with students to undergo training. It could be argued that students could be disadvantaged by a lack of understanding of educational teaching principles by clinical teachers who have not received training in practice education, particularly because students are assigned to multiple clinical teachers. There is clear justification for providing untrained clinical teachers at FHSUL with the necessary theoretical knowledge and skills to provide students with a consistent educational approach during clinical training. The importance of personal factors such as good communication skills, approachability, respect and support for students should be emphasised in clinical teacher education.

#### 4.2. Strengths and Limitations

The study had many strengths. This was the first study aimed at investigating students' perceptions of teaching and learning activities related to clinical education in Slovenia. The results of this study provide valuable insights into students' perspectives of clinical teaching and learning opportunities and the role and attributes of clinical teachers in the effectiveness of clinical education. One limitation is that we did not include the views of clinical teachers to gain insight into the similarities and differences between the perspectives of students and clinical teachers. Limitations of the study also include the fact that the study was only conducted at a single higher education institution. Had the study

been conducted at multiple higher education institutions in Slovenia, different phenomena and findings may have emerged.

### 5. Conclusions

In our study, demonstrations of patient management were found to be the most valuable activity for facilitating learning in the clinical setting from the students' perspective. However, demonstrations cannot exist in isolation but should be complemented by feedback and discussion. Feedback and discussion were seen by students as the second and third most important TLOs. Students perceived the most important role of the clinical teacher as a reflector/feedback and knowledge provider, mentor and demonstrator of techniques. Good communication skills, respect for students, approachability, interest in students and support for students were cited by students as the most important attributes of the clinical teacher. The fact that clinical teachers at FHSUL are not required to undertake formal training and often rely on the goodwill of physiotherapists in the clinical setting to work with students needs to change. FHSUL, which sends its students to specific practice settings, should consider providing these clinical teachers with the necessary training to provide students with an effective clinical placement experience.

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## Research

# Effects of Transcutaneous Electrical Nerve Stimulation in Patients with Fibromyalgia Syndrome – Literature Review

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**Abstract:**

**Introduction:** Fibromyalgia is a chronic disorder characterized by diffuse musculoskeletal pain, fatigue, depression and cognitive impairment. The aim of treatment is to alleviate symptoms, maintain physical function and improve quality of life. One of the recommended methods of a non-pharmacological approach is transcutaneous electrical nerve stimulation (TENS). The aim of the literature review was to determine the effects of TENS in fibromyalgia patients, focusing on the assessment of changes in pain intensity in relation to the minimal clinically important difference.

**Methods:** The literature search was conducted until the end of 2024 in the following databases PubMed, CINAHL and PEDro.

**Results:** Twelve studies were included in the review. Three studies looked at the immediate effects of TENS on pain and nine studies looked at the longer-term effects. The duration of the individual therapies ranged from 20 to 120 minutes, the stimulation frequency from 0.5 to 320 Hz and the intensity from pleasant to very strong but tolerable stimulation. Pain was assessed using a visual analogue scale and a numerical rating scale. **Discussion and conclusion:** The results indicate that TENS therapy is more effective when applied with sufficient intensity over multiple sessions rather than as a single treatment. While a one-time application may provide temporary pain relief, repeated treatments show more clinically significant effects. People with higher pain sensitivity tend to respond better to TENS. This therapy remains a promising non-pharmacological option for pain management in fibromyalgia, however, caution is needed in generalizing the results as the research has predominantly studied women.

**Keywords:** TENS; pain; clinically important difference; MCID

## 1. Introduction

Fibromyalgia is a chronic condition or syndrome characterized mainly by widespread musculo-skeletal pain. At the same time, systemic symptoms such as fatigue, sleep disturbances, morning stiffness, depression, anxiety, and cognitive impairments (e.g. forgetfulness, difficulties with concentration, attention, and memory) may occur (Clauw, 2009; Wolfe et al., 2010). The etiology is unknown, but there are some theories that descending pain inhibition and enhanced excitability of the central nervous system may change the processing of stimuli resulting in increased pain perception (Amer-Cuenca et al., 2023). This is why patients can experience diffuse hyperalgesia (increased sensitivity to painful stimuli), and allodynia (feeling pain in response to non-painful stimuli) can also be present (Clauw, 2009). The prevalence of fibromyalgia is between 0,2 % and 4,7 % in general population and predominantly affects women (female-male ratio of 3:1) (Amer-Cuenca et al., 2023; García-López et al., 2024).

Because pain is the main disabling symptom and the etiology is unknown the goals of treatment are symptom relief, maintaining function and improving quality of life (García-López et al., 2024; Macfarlane et al., 2017). The European League Against Rheumatism (EULAR) published revised recommendations for the treatment of fibromyalgia syndrome in 2016. They recommend an emphasis on starting treatments with non-pharmacological approaches, with active patient participation in self-management of the condition (Macfarlane et al., 2017).

A potential non-pharmacological pain control treatment is Transcutaneous Electrical Nerve Stimulation (TENS) frequently used to relieve acute and chronic pain. The device delivers alternating electrical current through electrodes placed on the skin (Vance et al., 2014; Amer-Cuenca et al., 2023). There are two main types of TENS used. High-frequency TENS, with a frequency of 50–200 Hz, is applied at a low intensity (below the motor threshold). Low-frequency TENS, with a frequency of 2–10 Hz, is applied at a higher intensity (above the motor threshold). A combination of these two is also used (Casale et al., 2012). Systematic reviews on the effectiveness of TENS for fibromyalgia syndrome have shown mixed results. Two systematic reviews (Johnson et al., 2017; Salazar et al., 2017), one with a meta-analysis (Salazar et al., 2017), could not make specific conclusions due to the lack of high-quality studies. However, more recent systematic reviews with meta-analysis have shown promising results (Amer-Cuenca et al., 2023; Batista de Aguiar et al., 2022; García-López et al., 2024). The most recent review, which included the largest number of clinical trials, found that TENS is effective in reducing pain and disability and improving physical quality of life in patients with FM. When compared to placebo and control, TENS was more effective, but it was not more effective than therapeutic exercise (García-López et al., 2024). One of the systematic reviews and meta-analysis discussed the TENS parameters (Amer-Cuenca et al., 2023). The recommended or appropriate parameters of TENS are considered as: high frequency (greater than 10 Hz and up to 200 Hz) or mixed frequency (switching between low and high frequency), with intensity set to produce a strong but comfortable sensation, or ideally the highest tolerable non-painful level. A minimum of 10 treatment sessions should be applied over the painful area. This approach led to a significant reduction in pain compared to other parameter combinations (Amer-Cuenca et al., 2023).

When evaluating outcome measures it is important to consider not only statistical significance, but also clinical importance of the change in scores. MCID is a minimal clinically important difference. That is the smallest level of change in a scale associated with a meaningful improvement in a patient. It is unique to each scale or questionnaire etc. and is important for interpretation of the results (Mease et al., 2011).

Pain intensity is most evaluated using the Visual Analogue Scale (VAS) or the Numerical Rating Scale (NRS). Both scales assess pain on a range from 0, indicating no pain, to 10, describing the worst pain imaginable (Ferreira-Valente & Pais-Ribeiro, 2011). On average, a reduction of one point or 15.0% on the NRS was found to represent the MCID for patients with chronic musculoskeletal pain. However, a reduction of 2 units or 33.0% was most strongly associated with the concept of "much better" improvement (Salaffi et al., 2004). The most used criteria for MCID in chronic pain, as established by Farrar and colleagues (2001) (who also included fibromyalgia patients), showed that a change of approximately 1.8 points or 28% in pain severity in NRS was most strongly associated with clinically important improvement. Similarly, Arnold and colleagues (2012) proposed that responder



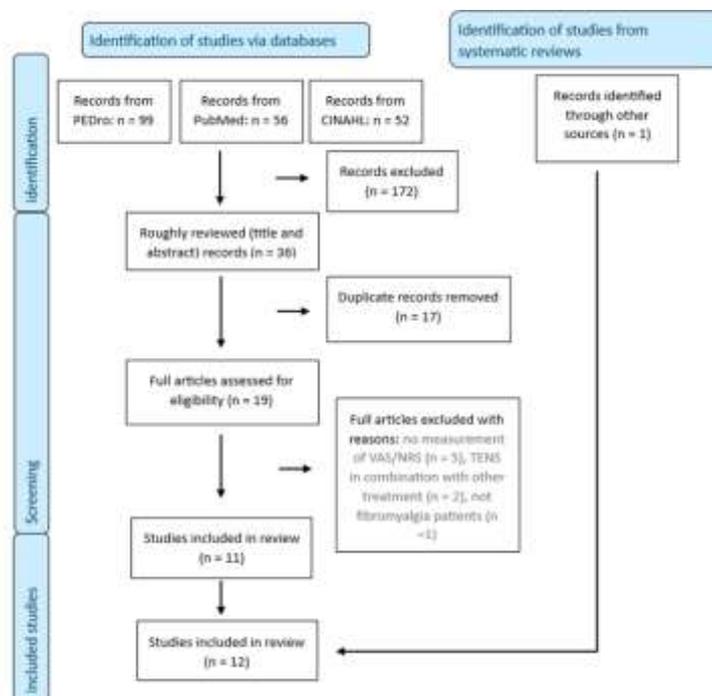
definition in patients with fibromyalgia requires  $\geq 30\%$  reduction in pain and a  $\geq 10\%$  improvement in physical function.

The purpose of this systematic review was to evaluate the effectiveness of TENS in patients with fibromyalgia, with a specific focus on assessing pain intensity changes in relation to the MCID.

## 2. Methods

Literature review has been conducted until the end of the year 2024 in databases: PubMed, CINAHL and PEDro with keywords: transcutaneous electrical nerve stimulation OR TENS and fibromyalgia.

Studies in English language that investigated the effects of TENS in patients with fibromyalgia and reported pain intensity measured by VAS or NRS were included. Reviews and studies that investigated combined effects with other physical modalities were excluded. The search method for articles is presented in more detail in **Figure 1**.



**Figure 1.** PRISMA diagram of the process of searching and collecting articles (Moher et al., 2009)

## 3. Results

A total of 12 articles were included in the review (Carbonario et al., 2013; Castro-Sánchez et al., 2020; Dailey et al., 2013; Dailey et al., 2020; Dailey et al., 2022; Di Benedetto et al., 1993; Jamison et al., 2021; Lauretti et al., 2013; Löfgren & Norrbrink, 2009; Riachi et al., 2023; Sunshine et al., 1996; Yüksel et al., 2019). In two studies they used a cross-over study design (Dailey et al., 2013; Löfgren & Norrbrink, 2009). Overall, there were 1022 (955 women, 26 men and 42 not specified) participants. Out of those, 457 (425 women, 11 men and 21 not specified) participants had active TENS treatment. Detailed study protocols, general results and quality of studies are reported in **Table 1**.

In three studies (Dailey et al., 2013; Riachi et al., 2023; Yüksel et al., 2019) they only applied TENS therapy for one session and looked for immediate effects of TENS on intensity of pain. Parameters of TENS treatment and results of the in-group change of pain intensity are reported in **Table 2** in greater detail.

In nine studies (Carbonario et al., 2013; Castro-Sánchez et al., 2020; Dailey et al., 2020; Dailey et al., 2022; Di Benedetto et al., 1993; Jamison et al., 2021; Lauretti et al., 2013; Löfgren & Norrbrink, 2009; Sunshine et al., 1996) they applied TENS for several sessions. Carbonario and colleagues (2013) investigated effects of TENS when applied during exercise. **Table 3** provides a detailed overview of the TENS treatment parameters and the in-group changes in pain intensity.

**Table 1.** Study characteristics and general results from studies

Author, year	Groups	Sample (gender, age)	General results	PEDro
Riachi et al., 2023	G: TENS (suit)	50 W (53.33 ± 7.08)	↓ pain (p < 0.001) immediately after treatment and 24 hours later	/
Dailey et al., 2013 *crossover	G1: TENS G2: PB-TENS G3: N-TENS	42 W, 1 M (49.2 ± 12.0)	No within group results reported ↓ pain (p < 0.05) in G1 compared to G2 and G3 for MEP, but not for PAR	9/10
Dailey et al., 2020	G1: TENS G2: PB-TENS G3: N-TENS	76 W (44.7 ± 14.3) 68 W (47.2 ± 12.6) 94 W (48.6 ± 11.8)	↓ pain (p < 0.05) in G1 ↓ pain (p < 0.05) in G1 compared to G2 and G3 for MEP and PAR	8/10
Dailey et al., 2022	G1: TENS G2: PB-TENS G3: N-TENS	103 W 99 W 99 W SG-O 52 <sup>Me</sup> (45 – 58) SG-NO 47 <sup>Me</sup> (35 – 56)	↓ pain (p < 0.05) in G1; regardless of the subgroup (SG-O or SG-NO)	8/10
Jamison et al., 2021	G1: TENS G2: PB-TENS	58 W, 4 M (52.3 ± 13.8) 53 W, 4 M (38.3 ± 13.1)	↓ pain (p < 0.029) in G1 compared to G2	6/10
Lauretti et al., 2013	G1: 2 TENS G2: 1 TENS, 1 PB-TENS G3: 2 PB-TENS	13 W (30 ± 12) 12 W, 1 M (32 ± 8) 9 W, 1 M (35 ± 8)	↓ pain (p < 0.05) within G1 and G2 No between group comparison	5/10
Sunshine et al., 1996	G1: massage G2: TENS G3: PB-TENS	10 W 10 W 10 W (49.8)	No change in pain (p > 0.05) in G2 No between group comparison.	2/10
Castro-Sánchez et al., 2020	G1: dry needling G2: TENS	28 W, 9 M (49.35 ± 5.82) 32 W, 5 M (47.84 ± 8.12)	↓ pain (p < 0.05) within G2 ↓ pain (p < 0.05) in G1 compared to G2	8/10
Di Benedetto et al., 1993	G1: pharmacotherapy G2: TENS	14 W, 1 M 15 W (51 ± 9.5)	No change in pain (p > 0.05) in G2 No between group comparison	4/10
Löfgren, Norrbrink, 2009 *crossover	G1: thermopack G2: TENS	32 W (41.5 ± 8.3)	↓ pain (p < 0.05) within G2 No between group comparison	6/10
Yüksel et al., 2019	G1: TENS G2: acupuncture G3: healthy control	21 (38.1 ± 11.3) 21 (44.6 ± 10.34) 21 (30.2 ± 6.5)	↓ pain (p < 0,001) within G1 No between group comparison	6/10
Carbonario et al., 2013	G1: exercise + TENS G2: exercise	14 W (52.9 ± 5.9) 14 W (51.9 ± 9)	↓ pain (p < 0,001) within G1	4/10

G – group, MEP – movement evoked pain, N-TENS – no TENS (control), PAR – pain at rest, PB-TENS – placebo TENS, SG-O – subgroup regularly taking opioids, SG-NO – subgroup not taking opioids regularly, ↓ - reduction



**Table 2.** Parameters of TENS and results of studies with only 1 treatment session

Authors, year	Characteristics of TENS treatment				Results			
	Parameters	Area	Duration	Frequency		Before	After	
Riachi et al., 2023	20 Hz; 25 – 170 µs 2mA	20 pairs of muscles	1 hour	1 session	VAS	immediately	6.20 ± 1.73 cm	2.36 ± 1.44 cm
						24 hours later	6.20 ± 1.73 cm	4.7 ± 1.97 cm
Dailey et al., 2013 *crossover	100 Hz; 200 µs max. tolerable	C7–T1 or L5–S1	60–75 minutes	1 session	VAS	30 minutes	PAR: 5.0 ± 0.5 cm MEP: 5.4 ± 0.4 cm	Δ = - 0.38 ± 0.26 cm Δ = - 1.1 ± 0.26 (6MWT)
Yüksel et al., 2019	70 Hz; 100 ms comfortable	T2–T7	20 minutes	1 session	VAS	immediately	5.19 ± 2.20 cm	2.86 ± 2.01 cm

MEP – movement-evoked pain, NRS – numerical rating scale, PAR – pain at rest, VAS – visual analog scale, 6MWT – 6-minute walking test, Δ – change (after – before)

**Table 3.** Parameters of TENS and results of studies with several treatment sessions

Author. year	Characteristics of TENS treatment				Results			
	Parameters	Area	Duration	Frequency		Before	After	
Sunshine et al., 1996	0.5 – 320 Hz	over painful areas	30 minutes	5 weeks. 2x/week (10 sessions)	VAS	7.5 cm	7.3 cm	
Castro-Sánchez et al., 2020	100 Hz; 200 µs tolerable	active and latent trigger points (5 muscles)	50 minutes (10 minute for each muscle pair)	6 weeks. 1x/week (6 sessions)	VAS		Δ = - 1.86 cm	
Lauretti et al., 2013	2 Hz and 100 Hz; 200 µs 60 mA	C7–T1 and L5	20 minutes	1 week. 2x/day. every day (14 sessions)	VAS	G1: 8.5 ± 2	4.3 ± 2 (Δ ~ 4 cm)	
						G2: 8.5 ± 1 cm	6 ± 1 cm (Δ ~ 2.5 cm)	
Löfgren & Norrbrink, 2009 *crossover	80 Hz strong but comfortable	painful area	at least 30 minutes	3 weeks. every day (21 sessions)	NRS	8.0 (IQR 6.0; 9.0)	6.3 (IQR 4.3; 7.3) 36 % responders	
Jamison et al., 2021	60 – 100 Hz; 290 µs strong but comfortable	upper part of calf	60 minutes	3 weeks. at least 2x/day (average 68.9 sessions)	NRS		Δ = - 1.83 ± 0.19 ≥30 % pain reduction: 28.4 % – 52.1 %; ≥50 % pain reduction: 12.6 % – 37.6 %	
Dailey et al., 2020	2–125 Hz; 200 µs max. tolerable	C7–T1 and lower back	at least 2 hours each day	4 weeks. every day (28 sessions)	NRS		PAR: Δ = - 1.9 MEP: Δ = - 1.8 (6MWT); Δ = - 1.6 (5STS) 44 % responders	
Dailey et al., 2022	2 – 125 Hz; 200 µs max. tolerable	C7–T1 and lower back	at least 2 hours every day (30 minutes per session)	4 weeks. every day (atleast 28 sessions)	NRS	(SG-O)	RAP: Δ = - 1.6 MEP: Δ = - 1.7 (6MWT); Δ = - 2.0 (5STS)	
						(SG-NO)	RAP: Δ = - 2.0 MEP: Δ = - 1.9 (6MWT); Δ = - 1.5 (5STS)	
Di Benedetto et al., 1993	80 – 100 Hz; 70 µs pleasant	4 painful points	80 minutes (20 minutes per point)	6 weeks. 5x/week (30 sessions)	VAS	5.6 cm ⊗	2 weeks: 4.8 cm ⊗ 4 weeks: 5.0 cm ⊗ 6 weeks: 4.6 cm ⊗	
Carbonario et al., 2013	150 Hz; 150 µs strong but comfortable	m. trapezius and m. supraspinatus	30 minutes during exercise	8 weeks, 2x/week (16 sessions)	VAS		Δ = - 2.0 ± 2.9 cm 30 % clinical gain	

MEP – movement-evoked pain, NRS – Numerical Rating Scale, PAR – pain at rest, SG-NO – subgroup not taking opioids regularly, SG-O – subgroup regularly taking opioids, VAS – Visual Analog Scale, ⊗ - score extracted from the graph, 5STS - Five Times Sit-to-Stand test, 6MWT – 6-Minute Walking test, Δ – chang

#### 4. Discussion

The current studies done in research of effects of TENS offer important insights for potential pain management in patients with fibromyalgia but also reveal several important limitations. In studies where they used only a one-session treatment using electrodes paravertebrally Dailey and colleagues (2013) and Yüksel and colleagues (2019) did report statistically significant pain reduction within group with active TENS. Interestingly movement evoked pain (MEP) showed improvement for more than 1 point on VAS, but with that not reaching completely the MCID, whereas pain at rest (PAR) didn't reach the statistical significance (Dailey et al., 2013). On the other hand, Yüksel and colleagues (2019) reported change of average pain enough for a clinically significant decrease of pain already in one session. Riachi and colleagues (2023) also showed clinically important improvement in pain reduction using a suit with electrodes that activated 20 pairs of muscles. Their study showed that a one-time treatment with low-frequency TENS with higher intensity resulted in a substantial pain reduction that lasted for up to 24 hours. This is probably because low-frequency TENS stimulate endogenous opioid release (Macedo et al., 2015). Despite these observations we cannot say for certain that one session of TENS treatment would give significant improvements in pain perception.

In contrast, studies with more frequent TENS sessions and appropriate parameters have demonstrated more favorable outcomes. In their study, Castro-Sánchez and colleagues (2020), although limited by an insufficient number of treatment sessions, still reported a statistically and clinically meaningful reduction in pain intensity, in contrast to Sunshine and colleagues (1996) who didn't find any significantly important difference in pain perception, even though experimental group had enough sessions, but lacked sufficient intensity of TENS. This suggests that the intensity of TENS plays a critical role in its efficacy, with low-intensity treatments, such as those only producing a tingling sensation, failing to deliver meaningful pain reduction. There is also important to note that this study is of low quality (Sunshine et al., 1996).

The studies that included the appropriate number of sessions and mostly appropriate parameters of TENS treatment (Carbonario et al., 2013; Dailey et al., 2020; Dailey et al., 2022; Di Benedetto et al., 1993; Jamison et al., 2021; Lauretti et al., 2013; Löfgren, Norrbrink, 2009) as suggested in systematic review and meta-analysis done by Amer-Cuenca and colleagues (2023), all reported a decrease in pain intensity for at least 1 unit or about 15 % improvement. All of them showed statistically important change except for one (Di Benedetto et al., 1993). The same study showed the lowest average change. This is the only study that applied lower intensity (pleasant feeling of TENS) and is also a lower quality study.

Löfgren & Norrbrink (2009) reported that the median score of pain was lower, and they also reported that 36 % of participants were considered responders, meaning they had a pain reduction of at least 2 units). Similarly, Dailey and colleagues (2020) reported that 44 % of people had 30 % less pain. Jamison and colleagues in 2021 also reported the percentage of participants considered to be responders. They found that 28.4 % of participants in the subgroup with lower pain sensitivity and 52.1 % of participants in the subgroup with higher pain sensitivity experienced a moderate reduction in pain severity (more than 30 %). When reporting substantial pain reduction (more than 50 %), 37.6 % of patients in the subgroup with higher pain sensitivity achieved this level of improvement. This suggests that people with a higher baseline pain sensitivity respond better to TENS treatment than those with lower sensitivity. An important study by Dailey and colleagues (2022) investigated the impact of taking oral analgesics on pain reduction when using TENS. According to their study, medication did not influence the outcome, meaning that there was a similar reduction in pain intensity regardless of whether participants regularly took medication or not.

Importantly, the studies conducted by Dailey and colleagues (2013; 2020) further highlight the cumulative effects of TENS treatment over time. While one of their studies found no improvement in pain at rest after a single TENS session (Dailey et al., 2013), their study from 2020 demonstrated a clinically meaningful reduction in pain at rest and movement-evoked pain after four weeks of treatment. The pain reduction was even greater after eight weeks of TENS treatment, illustrating that prolonged TENS use may lead to sustained im-

improvements in pain perception. This finding supports the notion that TENS treatment requires a sufficient duration to achieve clinically significant pain reduction, a conclusion also supported by systematic reviews and meta-analysis (Amer-Cuenca et al., 2023).

In this systematic review we see that when TENS is applied with sufficient intensity, there is some research supporting its ability to achieve not only statistically significant but more importantly, clinically meaningful reduction in pain. Generalization of these findings should be avoided due to predominantly female populations included in the referenced studies.

TENS offers a non-pharmacological option for managing chronic pain conditions, empowering patients to actively participate in their treatment. Moreover, as TENS devices are commercially available and not associated with serious side effects, patients can independently use them at home on a regular basis (Dailey et al., 2020; Jamison et al., 2021).

### 5. Conclusion

This systematic review highlights the potential of TENS as an option for pain management in patients with fibromyalgia. One-time treatments can have some effect on immediate pain relief, but more consistent results for a clinically meaningful reduction in pain intensity are seen when TENS is applied with sufficient intensity over several sessions. Given the promising evidence, further high-quality studies with larger sample size and diverse population (especially including more male participants) are needed to establish long-term efficacy in patients with fibromyalgia.

**Conflicts of Interest:** The authors declare no conflict of interest.

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Case report

# Pain Relief Treatment of Fresh Sacral Fracture for a Professional Snowboarder one Month before Participation in the Winter Olympic Games

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## Abstract:

Sacral fractures result from trauma, stress, or low-energy impacts, especially in weakened bones. These fractures often present with nonspecific symptoms and require advanced imaging for diagnosis. Treatment varies based on fracture severity, with options ranging from conservative to surgical approaches. Pain management can involve pharmacological and non-pharmacological methods, such as acupuncture. A multidisciplinary approach is essential for recovery, particularly for athletes, to ensure early mobilization and avoid complications. In the case of a professional snowboarder who sustained a sacral fracture before the 2022 Winter Olympics, a CT-guided pain block and acupuncture enabled him to compete pain-free just 32 days post-injury. This case highlights the importance of an individualized, multidisciplinary approach when managing fractures in high-performance athletes, balancing both the healing process and the need to return to competition.

**Keywords:** Sacral fractures; Pain relief; Professional sport; Treatment; Symptomatic improvement

## 1. Introduction

### 1.1. Sacral fractures and treatment

Sacral fractures can result from high-energy trauma, repetitive stress, or low-energy impacts, particularly in individuals with weakened bones. They are categorized into traumatic fractures, stress fractures (fatigue and insufficiency types), and low-energy fragility fractures. Traumatic sacral fractures often occur alongside pelvic ring injuries (Barber et al., 2023; Wagner et al., 2015). Stress fractures include fatigue fractures, caused by repetitive stress on a healthy bone (commonly in athletes), and insufficiency fractures, which result from normal stress on weakened bone, particularly in older adults with osteoporosis (Shankar et al., 2024). Low-energy fragility fractures, also linked to osteoporosis, may be associated with spinal disorders (Barber et al., 2023).

These fractures often present with nonspecific symptoms like diffuse pain in the lower back, pelvis, or buttock, leading to frequent misdiagnosis. While plain radiographs are usually ineffective, advanced imaging such as magnetic resonance imaging (MRI) or computed tomography (CT) scans significantly aids diagnosis (Shankar et al., 2024; Wagner et al., 2015). Treatment approaches depend on severity: non-operative management, such as rest and gradual activity resumption, works well for stable fractures, whereas surgical intervention is required for more severe cases. Despite their clinical relevance, sacral stress fractures remain under-recognized, with limited standardized treatment protocols and research into risk factors and outcomes (Barber et al., 2023; Shankar et al., 2024).

The AOSpine sacral classification system divides sacral fractures into three types: Type A fractures are stable and managed conservatively, Type B fractures involve instability and may require surgery depending on severity, and Type C fractures compromise spinopelvic stability, requiring surgical intervention. However, there is limited agreement at the subtype level, leading to variability in treatment approaches. Further research is needed to improve classification reliability and establish evidence-based treatment protocols (Camino-Willhuber & Urrutia, 2022; Lambrechts et al., 2023).

C0 sacral fractures, often classified as type 2 in the Fragility Fracture of the Pelvis system, can be managed nonoperatively if stable. If instability or discomfort prevents mobilization, surgical stabilization may be necessary. Sacroplasty and transiliac-transsacral screw fixation are minimally invasive options, while triangular osteosynthesis may be required for more complex fractures. For neurological issues, nonoperative management is typically effective, but surgical decompression may be required if symptoms persist. The treatment goal is early mobilization to avoid complications from prolonged bed rest (Lambrechts et al., 2023).

### 1.2. Pain relief treatment

Effective pain management in both elite athletes and the general population often requires a multidisciplinary approach that integrates pharmacological and non-pharmacological strategies. Wang (2018) highlights the critical role of advanced imaging-guided techniques—such as fluoroscopy, CT, and ultrasound—in delivering precise and safe interventional treatments for conditions like spinal pain, joint pain, and neuropathic pain. These methods facilitate targeted administration of therapies like corticosteroids or anesthetics, reducing risks of complications and enhancing efficacy.

Elite athletes frequently use medications such as non-steroidal anti-inflammatory drugs (NSAIDs), opioids, and anesthetics to manage pain. While these are effective for short-term relief, particularly for acute injuries or post-competition recovery, the potential for dependency and adverse effects from long-term opioid use necessitates a careful, balanced approach. Complementing pharmacological treatments with non-drug strategies—including physical therapy, exercise, psychosocial interventions, and attention to sleep and nutrition—is crucial for sustainable recovery and optimal performance (Hainline et al., 2018). Interventional techniques, including corticosteroid injections, play a significant role in both sports medicine and broader pain management contexts. For instance, ultrasound-guided injections for sacroiliac joint dysfunction or other joint-related pains, as noted by Wang (2018), offer comparable efficacy to fluoroscopy-guided approaches while minimizing radiation exposure. Similarly, Sandrasegaram et al. (2020) emphasize the value of conservative management approaches, such as physical therapy, ergonomic adaptations, and

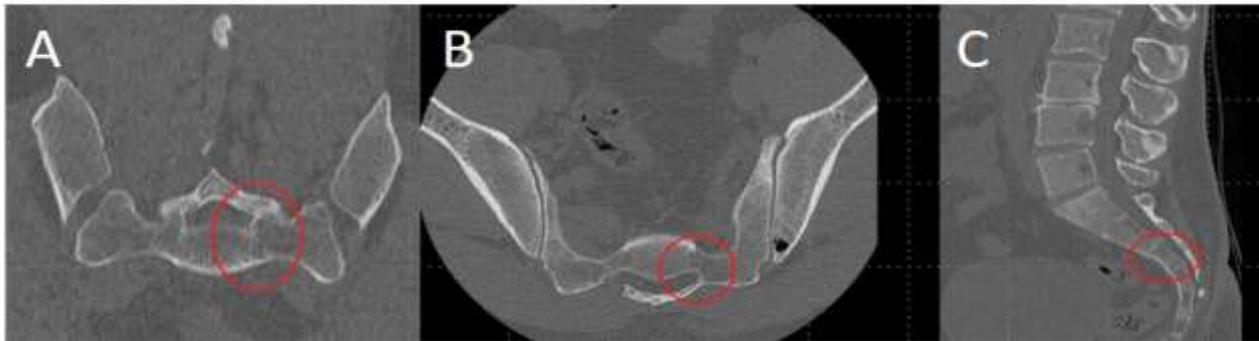
non-invasive interventions like ganglion impar blocks, in providing long-lasting pain relief with fewer risks. Newer therapies, such as platelet-rich plasma (PRP) injections, show promise but require further evidence to confirm their efficacy in various conditions (Hainline et al., 2018). Together, these approaches highlight the importance of an individualized, multidisciplinary care plan in effectively addressing both acute and chronic pain (Hainline et al., 2018; Sandrasegaram et al., 2020; Wang, 2018).

### 1.3. Supportive pain relief therapy

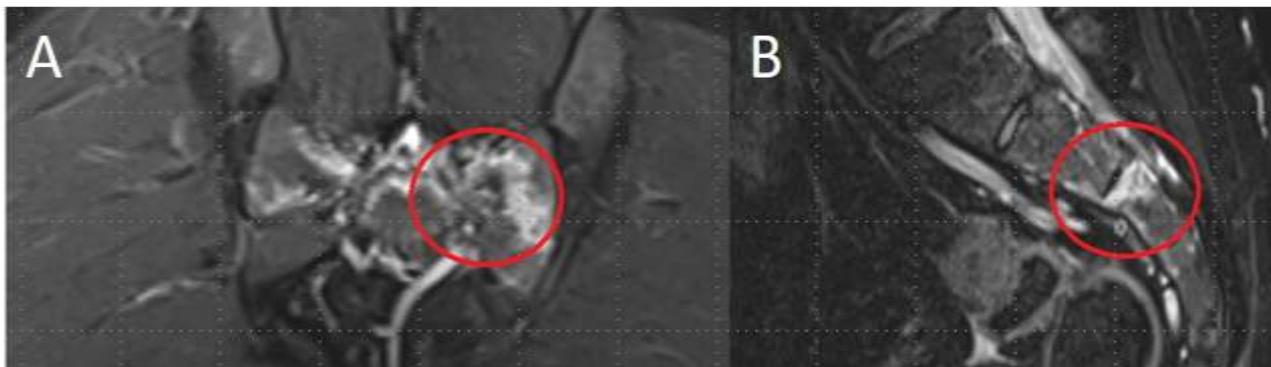
Acupuncture therapy has gained significant recognition as an effective non-pharmacologic treatment for managing acute pain, including in patients with fractures such as sacral fractures. Research suggests that acupuncture can play a crucial role in reducing pain levels in patients experiencing fractures, offering a valuable alternative to opioid medications, which are associated with risks of addiction and adverse side effects. Acupuncture works by stimulating specific points in the body, which may influence the nervous system, reduce inflammation, and promote the release of natural pain-relieving substances like endorphins. This process can contribute to reduced reliance on opioids, thus lowering the risk of opioid-related complications, including addiction and overdose (Nielsen et al., 2022). Numerous studies and systematic reviews have demonstrated acupuncture's efficacy in improving pain management, reducing the need for pain medication, and enhancing overall patient satisfaction, particularly in fracture recovery (Schug et al., 2020; Nielsen et al., 2022). Acupuncture has also been shown to reduce pain intensity and shorten recovery time, making it a promising complementary therapy in acute pain management. Moreover, acupuncture is considered a low-risk treatment with minimal side effects when administered by trained practitioners, which further highlights its potential as a safe and effective adjunct in fracture care. Given its therapeutic benefits and minimal adverse effects, acupuncture presents a valuable option for improving the management of acute pain in fracture patients. Additionally, acupuncture's ability to manage acute pain may allow for reduced opioid use and prevent long-term complications associated with opioid treatment (Schug et al., 2020).

## 2. Case presentation

In preparation for the 2022 Winter Olympic Games, a professional snowboarder came to the Emergency Centre of the Slovenj Gradec General Hospital on 11 January 2022. On 7.1.2022, he fell on the track. While checking the training track, he was hit by another snowboarder, his board slipped off, whereupon he fell on his back with great force onto a flat surface. Shortly after the fall, he realized that he was not in severe pain at rest but had a specific pain on the movement of one of his turns, which made it completely impossible for him to compete. Physiotherapy was started the same day after the fall. They used TECAR, massage, vacuum therapy, and laser. After physiotherapy, the pain in most of his back subsided, but it persisted mainly in his lower back and prevented him from snowboarding competitively. A CT scan was performed, which was suspicious of a fracture at the level of S3 (**Figure 1**). An MRI scan was performed within 24 hours and, after consultation with the radiologist, showed sacral edema at the S3 level with a fine transverse fracture line, which was consistent with the anatomical location of the patient's problems (**Figure 2**).



**Figure 1.** CT scan shows suspected S3 vertebral fracture (circled): A – Coronal CT image, B – Transverse CT image, C – Saggital CT image.



**Figure 2.** MRI showed marked edema of the bony body and posterior elements of S3 with a fine probable fracture (circled): A – Coronal T2-weighted STIR MRI, B – Saggital T1-weighted MRI.

With a non-dislocated stable sacral fracture, surgical treatment was not considered. After open consultation with the patient and recognition of the possible drawbacks of potent pain treatment in the context of successful healing of the fracture, the symptomatic (pain management) treatment was decided upon. A consortium of a trauma surgeon, an interventional radiologist, and an anaesthesiologist (pain management specialist) was gathered. A CT-guided block (0,25 % Chirocaine 3 ml (7,5 mg) + 16 mg Dexamethasone) at the injury site was performed on 17.1.2022. The Slovenia Olympic Committee's consent for the corticosteroid application (therapeutic exception) was obtained beforehand. Further pain treatment (acupuncture) was provided. The patient successfully qualified for the Olympic Games, where, although he did not repeat his previous successes, according to his testimony, he completed the competitive runs completely pain-free. The competition occurred on 8.2.2022, 32 days after the injury, 28 days after the diagnostics, and 22 days after the application of the blockade.

The patient did not develop a pain sequela later over time. He could participate in further sports events with no limitations, leading the authors of this article to believe that corticosteroid application in the setting of a fresh sacral injury did not detrimentally intervene with skeletal healing. No additional radiologic imaging was performed to confirm bony healing.

### 3. Discussion

Sacral fractures are rare and challenging to diagnose, often presenting with nonspecific symptoms that require advanced imaging for confirmation. Treatment typically involves non-operative approaches, though tailored strategies may be necessary for high-performing athletes (Barber et al., 2023; Shankar et al., 2024). Physiotherapists can contribute to the early diagnosis of sacral fractures by identifying specific patterns of pain and functional



limitations during biomechanical assessments and movement analysis. This can lead to timely referrals for imaging or further evaluation.

Sacral stress fractures are typically managed with conservative approaches, as seen in most reported cases. Treatment often includes rest, activity modification, physiotherapy to strengthen core muscles and improve flexibility, and nutritional supplementation to address any underlying deficiencies (Sandrasegaram et al., 2020; Silva et al., 2006; Vajapey et al., 2019). Silva et al. (2006) and Vajapey et al. (2019) describe instances where athletes, both amateur and professional, developed sacral stress fractures due to repetitive high-impact activities. These cases emphasize the success of non-operative treatments, which typically result in full recovery over several weeks through measures such as rest, cessation of high-impact activities, stretching exercises, and pain management with anti-inflammatory medications.

In managing sacral fractures, physiotherapy plays a significant role in reducing pain, improving functionality, and supporting recovery (Sandrasegaram et al., 2020). A comprehensive treatment plan, including active and passive ROM exercises, muscular strengthening, and various modalities like TECAR therapy, massage, vacuum therapy, and laser, can alleviate symptoms and promote gradual functional recovery. While physiotherapy helps alleviate pain and supports functional recovery, some cases may require advanced rehabilitation tools like antigravity treadmills and surgical interventions for severely displaced fractures. This integrated approach addresses both physical and psychological aspects, ensuring that athletes can return to their sport while minimizing long-term complications (Jaiswal et al., 2022).

In the notable case, a professional snowboarder with a sacral fracture received a CT-guided block and acupuncture, enabling him to compete pain-free in the Winter Olympics within 32 days of the injury. The reported case was a rare medical emergency where symptomatic improvement was attempted to establish a high functional requirement before the bone would physiologically heal completely. In doing so, the informed risk of the possible negative long-term health consequences of impaired healing of a sacral fracture was accepted. However, clinically, the patient remains without complaints to this day. The case underscores the need for a tailored, multidisciplinary, out-of-the-ordinary approach to managing fractures in high-performance athletes, where both the healing process and the urgency of returning to sport must be carefully balanced.

#### 4. Conclusions

The successful pain management of pain related to a non-dislocated traumatic sacral fracture in a professional snowboarder highlights the importance of a multidisciplinary approach. This case underscores the value of individualized strategies tailored to the demands of high-performance athletes.

**Conflicts of Interest:** The authors declare no conflict of interest.

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*Case report*

# Surgical Treatment of Chronic Post-Traumatic Hamstring Origin Pain with Tendon Transfer from the Unfused Ischial Tuberosity Apophysis to the Native Ischial Bone

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**Abstract:**

A case report of a 35-year-old female patient suffering from chronic hamstring origin pain after an acute strain injury is presented. The initial clinical examination demonstrated a significant tenderness of the left buttocks. The diagnostics showed a chronic displaced osteochondral fragment in the region of ischial tuberosity. The displaced fragment was interpreted as either an unfused apophysis or an unhealed childhood apophyseal avulsion fracture. The injury uncovered a “weak link” in the kinematic chain of posterior thigh and was therefore treated as a strain injury of pseudoarthrosis between the fragment and the pelvis. Conservative treatment, including physiotherapy and nonsteroidal anti-inflammatory medication, followed by a CT guided pain injection containing a corticosteroid and local anaesthetic failed to provide sufficient results. Therefore, operative treatment was recommended. Release and reattachment of the hamstring tendons from the fragment to the native os ischium, using three suture anchors, was performed with excellent results 15 months postoperatively. During the postoperative period and physical rehabilitation therapy, the patient gradually improved mobility, muscle strength and corrected her gait pattern. The patient was extremely satisfied with the surgical outcome and reported no discomfort or pain while performing daily activities.

**Keywords:** Ischial tuberosity; Chronic pain; Unfused apophysis; Tendon transfer; Rehabilitation.



## 1. Introduction

Proximal hamstring tendinopathy is the most common cause of chronic posterior thigh pain (Hunter & Speed, 2007). However, in some cases, the reason for pain can be found in other conditions with similar presentations, and the correct diagnosis is often missed due to the similarities in the mechanism of the injury and the area of pain (Dailey et al., 2013).

### 1.1. Ischial tuberosity and its ossification

The ischial tuberosity is the origin site of the muscles at the posterior compartment of the thigh, the hamstrings. The long head of biceps femoris and semitendinosus originate from the ischial tuberosity with a common tendon, while semimembranosus originates as a separate tendon laterally (Miller et al., 2007). Endochondral ossification, the process by which most of the skeleton is formed, involves the replacement of hyalin cartilage with bony tissue and proceeds at different rates in different bones (Mackie et al., 2007). Ossification of the ischial tuberosity typically starts between 13 and 15 years of age. During this process, due to the physiological properties of the hyaline cartilage, the ischial tuberosity is two to five times more fragile than the surrounding ligaments and tendons (Liu et al., 2018). The region of the ischial tuberosity has a limited capacity for force absorption, and an increase in mechanical load can result in avulsion fractures (Dailey et al., 2013).

### 1.2. Mechanism of the injury

Generally, these injuries are rare and occur frequently only in skeletally immature, active patients due to incomplete ossification (Liu et al., 2018). The mechanism of injury typically involves a powerful eccentric contraction of the hamstrings during sport activities involving rapid acceleration (e.g., sprinting and jumping (Liu et al., 2018; Tetsunaga et al., 2019)). As a result of delayed diagnosis or unsuccessful conservative treatment, some cases develop non-union, leading to chronic pain later in life (Ali et al., 2020). Asymptomatic unfused ischial tuberosity apophysis is rarely seen in adults, however, repeated micro-trauma during the process of growth and maturation can, in some cases, lead to non-union. Repetitive overload of the apophysis during sports activities can lead to apophysitis, and if the condition becomes chronic, the apophysis can remain unfused (Ali et al., 2020). The patient may later be symptom-free, while in some cases, the condition can lead to chronic pain in the posterior thigh (Ali et al., 2020; Dailey et al., 2013). Failure to treat the displaced fractures has been associated with pseudoarthrosis and proximal hamstring fibrosis, also leading to chronic pain and limitations on activities of daily living (Lui et al., 2018). An accurate diagnosis is, therefore, essential for proper clinical management.

### 1.3. Treatment

Conservative treatment, including modification of activities and anti-inflammatory medication is usually adequate for apophysitis (Ali et al., 2020). If diagnosed early and the size of fracture displacement is less than 2 centimeters, bony avulsions are also managed conservatively (Lui et al., 2018). Conservative treatment plan for avulsion fractures consists of four phases (**Table 1**) (Cereti et al., 2013).

**Table 1.** Conservative treatment plan for avulsion fractures (Cerreti et al., 2013).

Phase	Week	Conservative treatment plan
1	1 – 3	Resolution of pain, inflammation and acute symptoms. Rest, anti-inflammatory medication and passive mobilization are recommended.
2	3 – 8	Performing progressive agility and trunk stabilization exercises with low to moderate intensity for regaining mobility.
3	8 – 16	Performing progressive agility and trunk stabilization exercises with higher intensity.
4	16 – 24	Performing static stretching and isometric strengthening, followed by controlled eccentric and concentric contractions to increase strength and endurance.

Before returning to regular activity, the avulsed bone fragment must unite with the rest of the bone and the patient must regain full range of motion and adequate muscle strength. If the dislocation of the apophysis is greater than 2 centimeters or conservative treatment has not been successful, surgical reattachment may be considered (Liu et al., 2018). In cases of delayed diagnosis, unfused avulsed fragment usually forms a fibrous pseudoarthrosis with the rest of the bone (Liu et al., 2018). Excessive fibrous overgrowth during the healing process of avulsed ischial apophysis often causes symptoms later in life, such as discomfort while sitting and pain during activities (Schlonsky & Olix, 1972). Aggressive conservative treatment, including local corticosteroid injections and physiotherapy, is used to treat those symptoms. If the conservative treatment is unsuccessful, surgical dissection of fibrous tissue usually provides resolution of pain. In some cases, surgical reattachment of the avulsed fragment is performed (Liu et al., 2018; Schlonsky & Olix, 1972). Even though surgical treatment is considered effective in alleviating chronic pain, there is a lack of data published on these procedures (Liu et al., 2018). This report presents a case of successful surgical treatment of chronic post-traumatic hamstring origin pain with tendon transfer from the unfused ischial tuberosity apophysis to the ramus of the ischial bone.

## 2. Injury and treatment course

A 35-year-old female patient was presented to the emergency clinic after a slip in her bathroom, which resulted in an injury to her left leg. The patient described a typical stretch-type injury to the hamstrings, an extensive hip flexion with an extended knee (Danielsson et al., 2020). The initial clinical examination demonstrated a significant tenderness of the left ischial tuberosity. A computed tomography (CT) scan of the pelvis showed a displaced osteochondral fragment, interpreted as either an unfused apophysis or an unhealed childhood apophyseal avulsion fracture (**Figure 1a**). The patient's childhood history of gymnastics and athletics raised suspicion that sport-induced stresses could have contributed to either an acute trauma (which the patient does not recall) or an overuse injury that inhibited apophyseal fusion. Magnetic resonance imaging (MRI) of the pelvis confirmed a displaced osteochondral fragment, with all proximal hamstring tendons attached to it (**Figure 1b**). There was an effusion in the pseudoarthrosis between the fragment and the ischial bone, a sign of inflammation. Strain injury to the pseudoarthrosis was diagnosed. It seemed that the injury uncovered a developmental skeletal deficit.



**Figure 1a.** X-ray image from the time of the initial examination.

**Figure 1b.** MRI from the time of the initial examination. Note the effusion and accumulation of fluid within the pseudoarthrosis.

A senior trauma surgeon was consulted. Conservative treatment focused on alleviating pain was initiated, including physiotherapy and nonsteroidal anti-inflammatory medication. The patient was instructed to avoid movement that aggravated the symptoms. After three months, the patient was scheduled for control at the outpatient clinic. Conservative management did not provide sufficient results. The pain persisted, with increasing limitations in the activities of daily living. The patient reported having problems with sitting, walking and weight-bearing activities. Antalgic gait with decreased weight bearing on the affected site was observed. A pain management specialist was consulted and recommended a CT-guided pain injection. The injection containing a corticosteroid, and a local anaesthetic was injected to the affected area. The procedure provided temporary relief.

But, the pain rebounded, and the patient reported mild postprocedural irritation of the sciatic nerve, with an occasional tingling in the peripheral nerve field. Electromyography (EMG) demonstrated no abnormalities and MRI showed preserved nerve continuity. Ultimately, the pain management injection failed to provide sufficient results, and therefore, operative treatment was indicated.

The surgery was performed in a prone position under general anaesthesia. An incision was made along the gluteal crease, followed by the skin and subcutaneous tissue dissection. With the retraction of the gluteal muscles, the osteochondral fragment with attached hamstring tendons was exposed, and the sciatic nerve was identified. Fibrous tissue around the fragment was removed, while care being taken to avoid nerve damage. The release of hamstring tendons from the unfused fragment was performed. Reattachment of the tendons to the native os ischium was performed in the 60 degrees knee flexion, using three suture anchors. The postoperative anteroposterior radiogram of the pelvis and proximal femora is presented in **Figure 2**.



**Figure 2.** Postoperative anteroposterior radiogram of the pelvis and proximal femora.

Postoperatively, the left knee was immobilized in a hard frame brace, fixed at 60 degrees of flexion. At discharge, the patient was mobilized on crutches without load. Two weeks postoperatively, the brace was fixed at 30 degrees of knee flexion for an additional three weeks. The patient was instructed to avoid any active hip extension or knee flexion. Pain decreased significantly over time. Sciatic irritation faded. Six weeks postoperatively, full weight bearing was permitted. The patient started with physiotherapy, which included passive range of motion improvement exercises, ultrasound and laser to improve healing and electrotherapy for pain management. Seven weeks postoperatively, the patient improved significantly and could stand and walk without any discomfort. The range of hip motion was sufficient. A slight antalgic gait was still present. Nine weeks postoperatively, she underwent an intensive inpatient rehabilitation and balneotherapy at a local wellness center. Physiotherapy was focused on pain management, improving mobility and function. The two-week protocol consisted of active exercise, balance training, group hydro gymnastics, manual therapy, laser, and magnetotherapy. Pain was no longer present at discharge, and her gait pattern improved significantly. The range of hip motion increased in both flexion, extension, internal and external rotation and was in the range of normative

values for healthy adults. Fifteen months postoperatively, the patient was extremely satisfied with the surgical outcome and reported no discomfort or pain while performing activities of daily living.

### 3. Discussion and conclusion

This case report describes a patient suffering from chronic hamstring origin pain that started as an acute strain injury. The diagnostics uncovered a developmental skeletal disorder that was interpreted as either an unfused apophysis or an unhealed childhood apophyseal avulsion fracture. Generally, apophyseal avulsion fractures are rare and frequently occur only in skeletally immature, active patients. Individuals with undiagnosed, asymptomatic fractures are susceptible to recurrent injuries or complications. An unhealed avulsed fragment often forms a fibrous pseudoarthrosis with pelvis girdle and is prone to strain under heavy load. In the presented case, the patient injured the pseudoarthrosis between the hamstring origin and the pelvis. As a result, with muscle activation, the connection between the fragment and the ischium was subjected to strain, leading to the development of chronic pain. Initial conservative treatment, including physiotherapy, medication, and corticosteroid injections, did not alleviate the pain.

The literature confirms limited success of conservative treatment in chronic cases (Liu et al., 2018). Most authors agree that displacement greater of 2 centimeters is an indication for surgical intervention. Potential complications of conservative treatment include chronic pain, fibrosis at the origin of the hamstrings, sciatic nerve complications, muscle weakness, and inability to sit for a prolonged period (Liu et al., 2018; Schlonsky & Olix, 1972). Nevertheless, surgeons are often reluctant to operate in this anatomical region without extensive experience on account of the proximity of the sciatic nerve (Ali et al., 2020). To our knowledge, this is the first report describing surgical treatment of chronic hamstring origin pain with tendon transfer from the unfused ischial tuberosity apophysis to the native ischial bone. Suture anchor repair of the hamstrings was performed with direct exposure and protection of the sciatic nerve. Although the delayed diagnosis made it technically more challenging, excellent clinical, functional, and subjective results were achieved 15 months postoperatively. A comprehensive postoperative rehabilitation program contributed to improved mobility and muscle strength.

In conclusion, surgical intervention can address chronic issues caused by pseudoarthrosis and fibrous tissue overgrowth of hamstring origin, providing resolution of pain and functional deficits. The presented case demonstrates that hamstring tendon transfer with suture anchor repair to the ischial bone is an effective treatment option for chronic pain associated with unfused apophysis of the ischial tuberosity. When conservative treatment fails, surgical intervention can restore function and significantly improve quality of life.

**Conflicts of Interest:** The authors declare no conflict of interest.

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*Research*

# Ring-Shaped Lateral Meniscus – Rise Awareness and Avoid Unnecessary Surgery: A Case Report

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**Abstract:**

There are numerous meniscal anatomical variants, particularly prevalent in the East Asian population. These variants typically involve the lateral meniscus and are often asymptomatic. The ring-shaped meniscus is a relatively rare variant, with an incidence of 0.9 to 2.4% in the Asian populations. Magnetic resonance imaging (MRI) has a pivotal role in diagnosing meniscal and ligament injuries of the knee. Still, it has been difficult to distinguish a ring-shaped meniscus from a bucket-handle tear of a lateral meniscus or a central tear of a discoid lateral meniscus.

The subject was a woman in her 20s, reporting pain in her left knee after a mild sprain. The first MRI showed a small mediopatellar plica with a fissure of lateral femoral cartilage posteriorly and a massive bucket-handle rupture of the lateral meniscus with intercondylar incarceration of the fragment. During arthroscopic knee surgery, an intercondylar lateral meniscus fragment was visualized. The fragment could not be reponed due to a massive fibrous band attaching it to the joint capsule posteromedially. A congenital condition was suspected. Later, a follow-up bilateral MRI was performed, showing a symmetrical condition and confirming the presence of bilateral ring-shaped lateral menisci. The radiologist, however, still interpreted the finding as a bilateral incarcerated bucket-handle lateral meniscus tear.

Our case highlights the importance of knowing the plethora of meniscal variants to avoid unnecessary surgery.

**Keywords:** Ring-shaped meniscus; Lateral meniscus; Knee arthroscopy; Magnetic resonance imaging

## 1. Introduction

The menisci are fibrocartilages interposed between the femoral condyles and tibial plateau. In cross-section, they appear triangular and are fundamental for tibiofemoral congruence, playing an essential role in the kinetics of the knee (Faruch-Bilfeld et al., 2016). The medial meniscus is shaped like an open "C" and is attached to the medial capsule through the coronary ligaments. In contrast, the lateral meniscus is an incomplete, oval-shaped structure that lacks capsular attachments, making it more mobile than the medial meniscus (Iqbal et al., 2020).

There are numerous meniscal anatomical variants, and they are particularly prevalent in the East Asian population. These variants typically involve the lateral meniscus and are often asymptomatic (Tyler et al., 2010). The commonest of the variants is the discoid meniscus, with an incidence of about 16.6% in Asian populations and less than 5% in the Caucasian population (Lee et al., 2020). Other less common variants include the ring-shaped meniscus (RSM), abnormal bands of the lateral meniscus and double-layered meniscus. The RSM is relatively rare, with an incidence of 0.9 to 2.4% in the Asian populations (Kim et al., 2006). Noble (1975) was the first to document a ring-shaped lateral meniscus as an incidental finding during autopsy. Monllau et al. (1998) further described it as a meniscus with the anterior and posterior horns connected by an inter-horn meniscal bridge. While meniscal malformations are generally considered congenital, some cases may result from meniscal regeneration following knee operations (Nagashima et al., 2019). Magnetic resonance imaging (MRI) has a pivotal role in diagnosing meniscal and ligament injuries of the knee (Faruch-Bilfeld et al., 2016). However, certain anatomical variants can be confused with pathological conditions, such as a displaced bucket-handle tear (Kim et al., 2006). Diagnosing a ring-shaped lateral meniscus on MRI is particularly challenging because its appearance, with a centrally located fragment, often resembles a displaced meniscal tear (Atay et al., 2002).

This case report aims to raise awareness among healthcare professionals about possible rare congenital conditions affecting the lateral meniscus, which could help prevent unnecessary surgical interventions.

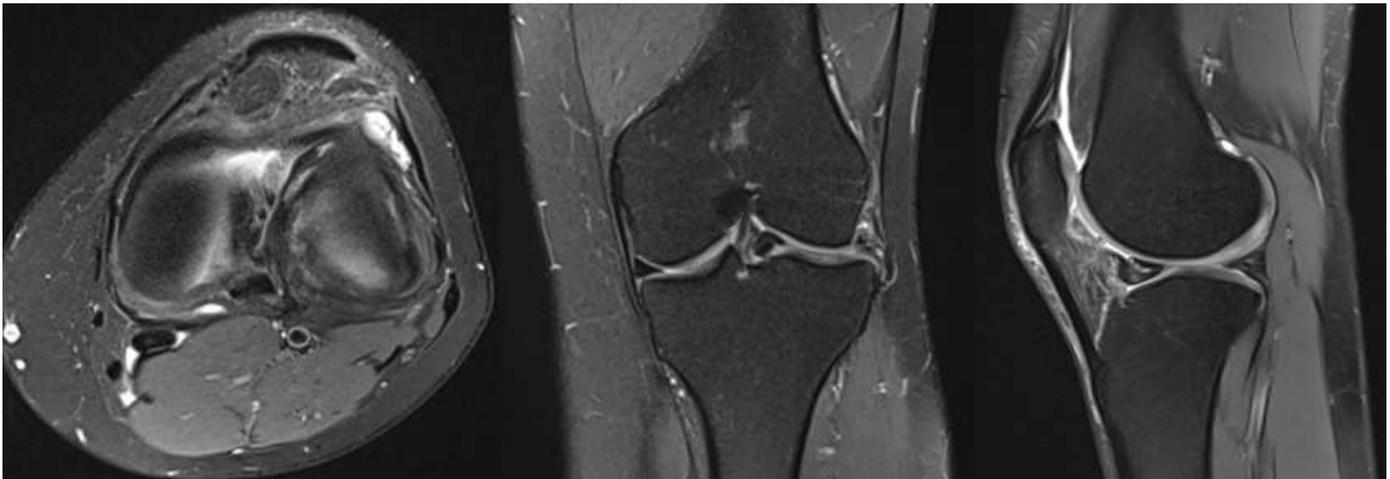
## 2. Case presentation

A woman in her 20s was referred to our outpatient clinic complaining of pain in her left knee. She reported a mild sprain of the knee. Previous major trauma to the knee was not reported. Magnetic resonance imaging (MRI) revealed a small mediopatellar plica with fissure of lateral femoral cartilage posteriorly and a massive bucket-handle rupture of the lateral meniscus with intercondylar incarceration of the fragment. The medial meniscus, anterior and posterior cruciate ligament, collateral ligaments, patellar ligament and quadriceps tendon showed no pathology.

On physical examination, the knee range of motion was preserved, and no instability of the knee joint was observed. Pain was occasional. The patient reported pain on palpation on the lateral femorotibial joint line and directly on the patellar ligament. Due to minimal clinical symptoms, a senior surgeon was consulted to discuss the indications for possible treatment strategies due to a low-symptomatic clinical presentation of a suspected bucket-handle tear. A knee arthroscopy was recommended. Awaiting the surgery, reduction and modification of physical activity was advised.

## 3. Treatment

An arthroscopic knee surgery was performed about one month after the outpatient visit. During the arthroscopic examination the intercondylar lateral meniscus fragment was visualised. As the fragment could not be repositioned laterally, partial tissue resection connecting the fragment posteromedially and popliteally was attempted. As the resection was being performed, the extent of the posterior fibrous band was appreciated. Realizing that an extensive tissue resection would be needed to attempt a successful reposition of the fragment laterally, the surgeon did not carry the procedure through. Given the patient had minimal preoperative issues, it was decided not to perform a large-scale resection that could alter the patient anatomy and potentially cause difficulties.



**Figure 1.** MRI transverse (left), coronal (centre) and sagittal (right) plane view.

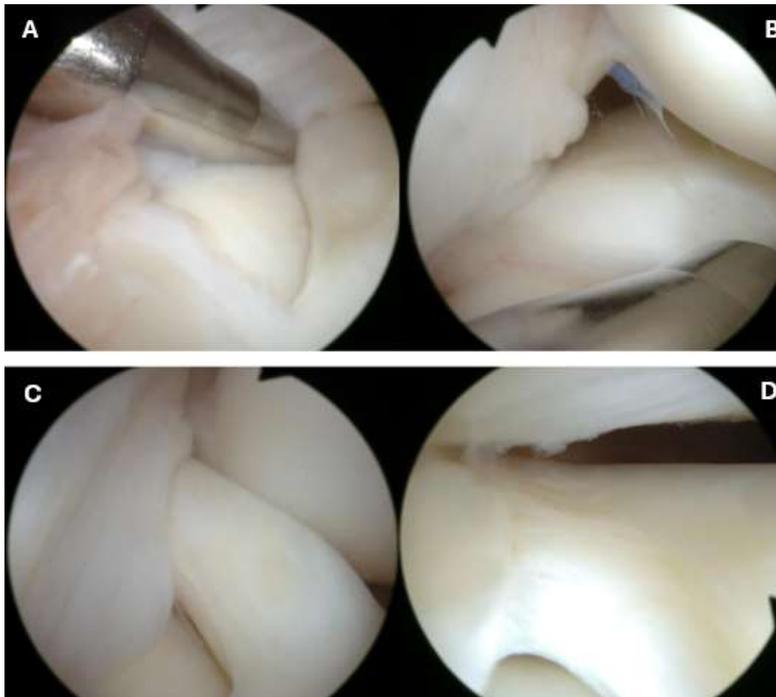
The patient recovered quickly after the arthroscopy and was discharged from the hospital the following day. She received standard analgesics and anticoagulants. Postoperative course was uneventful. Physiotherapy was suggested.

Postoperative rehabilitation focused on improving the range of motion and strengthening the thigh muscles until normalization. The patient immediately began light isometric contractions of the quadriceps muscle combined with Biopton therapy for 2 weeks. After suture removal, she received TECAR therapy four times with additional manual lymph drainage. She was taught scar massage and self kinesiotape application for circulatory/lymphatic drainage. She used a Compex Sport Elite muscle stimulator daily for initial muscle strengthening. From week one to two, the program used was Disuse atrophy while doing isometric quadriceps femoris muscle contractions. From week three to six she advanced to Muscle growth, and six to nine to Reinforcement. Progressively, she increased the difficulty of exercises from isometric contractions to closed kinetic chain exercises, like squats. For pain management she used Modulated TENS when needed. For additional muscle strengthening, conditioning, and a gradual return to sports, she worked with a kinesiologist twice a week for two months.

#### 4. Outcome and additional diagnostic procedures

About three months after the arthroscopy, the patient returned to the clinic for a follow-up. She reported pain during cross-leg sitting and on palpation on the anterolateral joint line and arthroscopic scars. On detailed medical history, she reported episodes of pain in both knees since childhood.

The arthroscopic view of the operated lateral meniscus (**Figure 2**) differed from the usual bucket-handle ruptured meniscus. The attachment of the meniscus to the popliteal capsule was uncommon. As well, there were only minor findings suggesting a traumatic event, therefore a congenital condition was suspected. A centrally ruptured discoid lateral meniscus seemed a possible clinical hypothesis. The patient was referred to bilateral knee MRIs. The MRI showed symmetrical condition, while reporting no previous trauma of the right knee, which was completely asymptomatic. The radiologist reported a bilateral bucket-handle tear with an intercondylar incarceration of the fragment. A ring-shaped lateral meniscus was diagnosed after conducting literature research and further consultation with radiologists.



**Figure 2.** Arthroscopic view of the ring-shaped lateral meniscus. **A:** anterolateral view, **B:** meniscus continues posteriorly, **C:** anteromedial view, **D:** anterolateral to posterolateral view (meniscus continues medially).

### 5. Discussion

The lateral meniscus is morphologically more prone to variations, predominantly found in Asian populations (Lee et al., 2020). The most frequent meniscal variant is the discoid meniscus, classified by Watanabe & Ikeuchi (1969) into three types: complete discoid meniscus, incomplete discoid meniscus and Wrisberg type meniscus. In 1998, Monllau et al. proposed the addition of RSM as a fourth type, leading to the development of the modified Watanabe classification system.

RSM is generally considered to be a congenital variation of the lateral meniscus, since patients usually present no symptoms or history of significant trauma (Arnold & Van Kampen, 2000; Soejima et al., 2013; Tyler et al., 2010). Additionally, Le Minor (1990) also identified RSM in primates, such as gorillas, further supporting its congenital origin. However, there are reports of symptomatic RSM cases, which are usually accompanied by meniscal cysts (Atay et al., 2002; Arnold & Van Kampen, 2000; Koukoulias & Papastergiou, 2011), medial plicae (Asadullayeva et al., 2024), meniscal tears (Kim et al., 2006) or other concurrent abnormalities or malformations of the knee (Rahij et al., 2024). In our case, MRI similarly revealed a small medial plica and meniscal cyst, indicating that the symptoms were not necessarily produced by the meniscus, as a RSM was also present in the other, asymptomatic, knee.

On the other hand, we can find literature suggesting regenerative formation of RSM after partial meniscectomy (Nagashima et al., 2019), anterior cruciate ligament (ACL) reconstruction (Fujii et al., 2017), and partial resection of a discoid meniscus with ACL reconstruction (Soejima et al., 2013). Similarly, cases of regenerative formation of a discoid meniscus have been reported in children (Bisicchia & Tudisco, 2013). Although the exact mechanism of regeneration is unknown, most authors suggest it is influenced by active growth plates that promote tissue repair (Nagashima et al., 2019) or the environment after ACL reconstruction, where drilling debris may contain mesenchymal stem cells and growth factors that promote intraarticular tissue formation (Fujii et al., 2017; Soejima et al., 2013).

Lateral meniscus variants are mostly detected on MRI (Lee et al., 2020). Still, it has been difficult to distinguish a RSM from a bucket-handle tear of the normally C-shaped lateral meniscus (Kim et al., 2006) or a central tear of a discoid meniscus (Lee et al., 2020). MRI signs that could aid in the recognition of a RSM are typically the presence of "the mirror image sign" and "the central bow tie sign" (Iqbal et al., 2020).

In differential diagnosis, we should also remember that bucket-handle ruptures are more common in the medial meniscus due to its firm attachment to the capsule and consequent greater stability (Niitsu et al., 2003). As bucket-handle ruptures of the medial meniscus have a predilection of 76% (Ververidis et al., 2006), the involvement of the lateral meniscus should also raise suspicion of congenital meniscal variants (Fujikawa et al., 2002).

## 6. Conclusions

When interpreting MRI images, it is essential to be aware of the signs mentioned previously to avoid unnecessary surgery (Asadullayeva et al., 2024). Nevertheless, a detailed history and note of trauma should be taken, as well as medical symptoms evaluated when interpreting the MRI findings (Kim et al., 2006). The final decision whether to operate should therefore not be made exclusively on the MRI.

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*Research*

# What are the Endurance Times of the Trunk Muscle Endurance Tests on the Roman Chair: A Preliminary Normative Study

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**Abstract:**

Sufficient trunk muscle endurance and an appropriate ratio between endurance times of the major trunk muscle groups are important factors in the prevention of low back pain. Trunk muscle endurance tests on the 45° Roman chair can be used as an alternative to the standard tests, but there is a need to collect normative data for these tests.

The aim of this preliminary normative study was to assess the endurance times of the trunk muscles and the ratio between endurance times of these trunk muscles during endurance tests on the Roman chair.

One hundred and six healthy participants aged 16-76 years (mean age: 36.9±11.7 years) without recent injuries or musculoskeletal disorders participated in our study. Trunk muscle endurance was tested on the Roman chair in four test positions: an endurance test for the trunk extensors, the trunk flexors and the lateral trunk muscles on the right and left side. For each test, the endurance time was recorded in seconds and the four trunk muscle endurance ratios were calculated for each participant.

The mean endurance time in the trunk extensors endurance test was significantly ( $p = 0.03$ ) longer in women than in men. In addition, the ratios between the lateral trunk muscles and the extensors also differed significantly between women and men ( $p < 0.05$ ).

The preliminary results on the normative endurance times for the trunk muscles in the Roman chair tests indicate some differences in muscle endurance between the genders. A larger sample of participants is required to determine normative values.

**Keywords:** Endurance tests; Roman chair; Physical performance; Spine stabilisation; Core muscles

## 1. Introduction

Back and abdominal muscle endurance is one of the key elements of physical performance that can affect spinal health by providing good/poor stability of the lower back and thus preventing injury (Panjabi, 1992; Kisner & Thorp, 2018). Therefore, sufficient trunk muscle endurance and an appropriate ratio between endurance of the major trunk muscle groups, i.e. the trunk extensors, trunk flexors and lateral trunk muscles, are important factors in the prevention of low back pain (Panjabi, 1992; McGill et al., 1999; McGill, 2016).

As McGill (2016) has already emphasised, optimal tests that isolate the trunk extensors, flexors and lateral trunk muscles are difficult to find. The endurance tests proposed by this author are 1) the extensor endurance test - a modification of the Biering-Sorensen test (Biering-Sørensen, 1984), 2) the flexor endurance test - sitting posture with a 60° angle to the floor and 3) the side bridge test (McGill, 2016). However, many authors emphasised that the endurance of other muscles (e.g. hip and shoulder muscles, etc.) (Pagé et al., 2011; Juan-Recio et al., 2022), shoulder pain or weakness (Ledoux et al., 2012; Pagé & Descarreaux, 2012) and the different type of kinetic chain (Tuff et al., 2020) may contribute to the performance of the above test positions.

The trunk muscle endurance tests on the 45° Roman chair showed some comparable characteristics and some potentially more feasible characteristics than the standard tests, i.e. the McGill tests (Petrič et al., 2022). Considering the characteristics and usefulness found in our previous study (Petrič et al., 2022), the Roman chair endurance tests can be used as an alternative to the standard tests mentioned above: All four trunk muscle groups are tested in a unified body position, a unified inclination angle and in an open kinetic chain. They have been shown to be easier and quicker for the evaluator to perform (no additional preparation or stabilisation is required, etc.), and in terms of perceived exertion by the participants, these tests are slightly easier to perform compared to the McGill tests (Petrič et al., 2022).

As the test positions of the Roman chair tests have only been used in a few studies so far (Ledoux et al., 2012; Pagé & Descarreaux, 2012; Petrič et al., 2024), there is a need to develop a normative database for these tests. The aim of this preliminary normative study was to assess the endurance times and the ratio between endurance of the major trunk muscle groups during the Roman chair tests.

## 2. Material and Methods

The study was designed as a preliminary normative study with a simple measurement of endurance times in four trunk muscle endurance tests on the Roman chair. The measurements were performed at the Faculty of Health Sciences of the University of Ljubljana (Slovenia). This study was approved by the National Medical Ethics Committee of the Republic of Slovenia (0120-220/2019/6).

### 2.1. Study sample

One hundred and six participants aged 16 to 76 years without current musculoskeletal injuries or diseases participated in our study. They were invited via electronic media and chain referral sampling in various research projects (PhD research, HUD 2024 project of the Faculty of Health Sciences, University of Ljubljana etc.). The inclusion criteria were a) healthy participants without musculoskeletal injuries or other conditions, that could be a contraindication for the muscle endurance test, b) and without low back pain at the time of enrolment in the study. All participants were volunteers and provided written informed consent prior to participation in this study.

### 2.2. Study instruments and procedures

All participants completed questionnaires regarding their demographic data and health status (Jakovljevič et al., 2017).

The endurance time (in seconds) of the four major trunk muscle groups was measured: trunk extensors (EX), trunk flexors (FL), right lateral trunk muscles (R-LM) and left lateral trunk muscles (L-LM). All four tests were performed on the 45° Roman chair, with the test positions, test procedures and calculation of trunk muscle endurance ratios following the protocols previously described in Petrič et al. (2022) (referred to as "B tests" in this article).

2.3. Study statistics

The statistical analysis consisted of calculating mean endurance times (in seconds) and ratio between endurances of the major trunk muscle groups with standard deviations. The endurance times in each trunk muscle group were compared between women and men (t-test for unrelated samples or Mann-Whitney U-Test for unrelated samples). Statistical significance was set at  $p \leq 0.05$  for all analyses. Data analysis was performed using an Excel programme (Microsoft Corporation, Washington, United States) and IBM SPSS Statistics 29 (IBM, New York, United States).

3. Results

One hundred and six participants aged 16-76 years (mean age:  $36.9 \pm 11.7$  years) took part in a study. In terms of gender, participants differed significantly in mean body height and mass ( $p < 0.001$ ; **Table 1**).

**Table 1.** Demographic data of participants by gender (mean  $\pm$  standard deviation).

	n	Age (years)	Body height (m)	Body mass (kg)	BMI (kg/m <sup>2</sup> )
Women	86	$37.1 \pm 12.5$	$1.67 \pm 0.05$	$64.1 \pm 10.3$	$23.0 \pm 3.7$
Men	20	$36.3 \pm 7.1$	$1.80 \pm 0.06$	$78.8 \pm 11.1$	$24.3 \pm 2.7$
p		0.79	$< 0.001^*$	$< 0.001^*$	0.10
All	106	$36.9 \pm 11.7$	$1.69 \pm 0.07$	$66.9 \pm 12.0$	$23.3 \pm 3.6$

n, number of participants; BMI, body mass index; p, Asymptotic sig. (2-sided test); \*, statistically significant differences

The mean endurance time in the EX endurance test was significantly ( $p = 0.03$ ) longer in women than in men (**Table 2**). The ratios between each side LM and EX also differed significantly between women and men ( $p < 0.05$ ) (**Table 2**).

**Table 2.** Preliminary results of endurance times (in seconds) and endurance ratios for women, men and all participants together (mean  $\pm$  standard deviation).

Gender	Endurance times (s)				Endurance ratios			
	EX	FL	L-LM	R-LM	FL:EX	R-LM: L-LM	L-LM: EX	R-LM: EX
Women (n = 86)	$362.7 \pm 247.9$	$193.6 \pm 181.9$	$145.5 \pm 71.0$	$127.4 \pm 60.3$	$0.63 \pm 0.50$	$0.91 \pm 0.26$	$0.47 \pm 0.20$	$0.42 \pm 0.19$
Men (n = 20)	$248.4 \pm 70.4$	$202.9 \pm 154.9$	$140.9 \pm 55.2$	$131.3 \pm 57.6$	$0.78 \pm 0.44$	$0.94 \pm 0.23$	$0.58 \pm 0.15$	$0.53 \pm 0.13$
p	0.03*	0.76	0.99	0.62	0.06	0.61	0.02*	0.01*
All (n = 106)	$341.1 \pm 229.5$	$195.3 \pm 176.4$	$144.7 \pm 68.1$	$128.1 \pm 59.6$	$0.66 \pm 0.49$	$0.92 \pm 0.25$	$0.49 \pm 0.20$	$0.44 \pm 0.19$

SD, standard deviation; n, number of participants; EX, endurance of trunk extensors; FL, endurance of trunk flexors; L-LM, endurance of lateral trunk muscles (left side); R-LM, endurance of lateral trunk muscles (right side); p, Asymptotic sig. (2-sided test); \*, statistically significant differences



Mean endurance times and endurance ratios by age group are listed for women in **Table 3** and for men in **Table 4**.

**Table 3.** Preliminary results of endurance times (in seconds) and endurance ratios for women (n = 86) by age group (mean ± standard deviation).

WOMEN (n = 86)		Endurance times (s) mean ± SD				Endurance ratios mean ± SD			
Age group (years)	n	EX	FL	L-LM	R-LM	FL:EX	R-LM: L-LM	L-LM: EX	R-LM: EX
16-25	16	466.9 ± 325.4	172.3 ± 105.1	148.5 ± 50.9	117.8 ± 41.1	0.48 ± 0.33	0.83 ± 0.26	0.40 ± 0.19	0.32 ± 0.14
26-35	29	357.2 ± 260.0	209.4 ± 254.5	135.1 ± 65.2	125.3 ± 54.2	0.60 ± 0.44	0.95 ± 0.22	0.45 ± 0.18	0.42 ± 0.19
36-45	18	285.1 ± 112.0	201.7 ± 110.6	120.6 ± 41.5	110.7 ± 43.0	0.87 ± 0.69	0.94 ± 0.23	0.47 ± 0.21	0.44 ± 0.21
46-55	13	436.9 ± 238.1	193.1 ± 168.5	215.2 ± 96.8	180.9 ± 78.5	0.55 ± 0.46	0.89 ± 0.29	0.57 ± 0.22	0.47 ± 0.19
> 56	10	254.8 ± 116.5	167.6 ± 120.1	125.4 ± 53.9	108.9 ± 60.2	0.65 ± 0.31	0.88 ± 0.32	0.55 ± 0.20	0.46 ± 0.20

n, number of participants; SD, standard deviation; EX, endurance of trunk extensors; FL, endurance of trunk flexors; L-LM, endurance of lateral trunk muscles (left side); R-LM, endurance of lateral trunk muscles (right side)

**Table 4.** Preliminary results of endurance times (in seconds) and endurance ratios for men (n = 20) by age group (mean ± standard deviation).

MEN (n = 20)		Endurance times (s) mean (SD)				Endurance ratios mean (SD)			
Age group (years)	n	EX	FL	L-LM	R-LM	FL:EX	R-LM: L-LM	L-LM: EX	R-LM: EX
16-25	1	269.0 ± 0.0	68.0 ± 0.0	103.0 ± 0.0	84.0 ± 0.0	0.25 ± 0.0	0.82 ± 0.0	0.38 ± 0.0	0.31 ± 0.0
26-35	8	243.4 ± 63.5	194.6 ± 137.4	135.8 ± 44.1	130.3 ± 56.0	0.77 ± 0.41	0.96 ± 0.20	0.56 ± 0.12	0.53 ± 0.12
36-45	9	255.1 ± 72.3	248.8 ± 164.8	157.4 ± 62.7	144.1 ± 60.1	0.91 ± 0.43	0.94 ± 0.26	0.63 ± 0.16	0.55 ± 0.10
46-55	2	227.5 ± 80.5	96.5 ± 10.5	106.0 ± 15.0	101.0 ± 4.0	0.50 ± 0.22	0.98 ± 0.18	0.51 ± 0.11	0.51 ± 0.20
> 56	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

n, number of participants; SD, standard deviation; EX, endurance of trunk extensors; FL, endurance of trunk flexors; L-LM, endurance of lateral trunk muscles (left side); R-LM, endurance of lateral trunk muscles (right side); N/A, results not yet available

#### 4. Discussion

The results of a preliminary normative study on the endurance times of trunk muscle endurance tests on the Roman chair indicated that, with the exception of the EX endurance test, women and men have a comparable mean endurance time. Therefore, the endurance ratios of the trunk muscle groups also differed between men and women for LM and EX (the ratios L-LM:EX and R-LM:EX), but not for the other two ratios. The lower number of male participants must be taken into account.

There are only a few data sets that can be compared with the data from this study. Pagé & Descarreaux (2012) reported slightly shorter mean endurance times in the L-LM endurance test (96.7 (24.9) s) and R-LM endurance test (97.2 (21.5) s) in healthy participants (n = 22; mean age: 24.55 (5.00) years). Compared to the results of Ledoux et al. (2012), in which healthy older adults participated (n = 32; mean age: 67.25 (5.13) years), the participants in our study (> 56 years old women) achieved a longer mean endurance time in the EX endurance test (mean endurance time in their study: 221.61 (108.6) s) and a similar mean endurance time in the LM endurance test (mean endurance time in their study: 127.32 (74.32) s). No study comparing the results of the FL endurance test could be found in the available literature.

The results of our study show some similar findings to the results of another preliminary normative study by McGill et al. (1999), in which they strive to assess endurance times in the tests proposed by McGill (McGill, 2016). Despite different body positions in both groups of endurance tests, we all found that women had longer endurance times than men in EX (p = 0.03), and that men were able to maintain LM tests for a longer period (p < 0.05) of their EX endurance times (i. e., LM:EX ratios) than women (e. g., for the LM test in the left side: 58% vs. 47% of their EX test). As reported in our previous study (Petrič et al., 2022), longer endurance times are generally observed for at least three out of four Roman chair tests compared to the McGill tests (McGill et al., 1999).

The study has some limitations. The first limitation of this preliminary normative study is the small and unevenly gender distributed sample of participants. In our study, there were more female than male participants. In addition, the sample of participants was unevenly distributed with respect to age, with most participants being between 20 and 45 years old. A larger and more homogeneous sample of participants with respect to gender and age is needed to continue this normative study.

This is the first study to collect normative data on Roman chair endurance testing.

#### 5. Conclusions

The preliminary results on the normative endurance times for the trunk muscles in Roman chair tests show some differences between the sexes (especially in the trunk EX endurance). A larger sample of participants is required to determine normative values, particularly a larger sample of male participants and participants under 20 and over 45 years of age.

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*Review*

# Destigmatization of Erectile Dysfunction

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**Abstract:**

Erectile dysfunction (ED) is a sexual dysfunction that significantly affects men's quality of life and is often associated with a stigma that makes it difficult to access appropriate treatment. This stigma, which is accompanied by feelings of shame and fear of judgment, leads to social isolation and increases psychological distress. ED is often linked to social norms that associate masculinity with strong sexual performance, which further complicates treatment. Comprehensive strategies to reduce the stigma associated with ED are of paramount importance. Public health campaigns that raise awareness of the nature of ED and promote open communication about sexual health are essential to dispel myths and reduce the shame associated with this condition. In addition, incorporating sexual health into routine medical examinations allows for early detection and treatment of ED, which helps to reduce stigma. A culturally sensitive approach that takes into account the specific values in different social settings is also crucial in combating stigma, especially in cultures where sexual topics are taboo. When healthcare providers are trained to address ED in a non-judgmental way, men can seek help without fear of criticism. Comprehensive strategies, including awareness campaigns, education and improved access to treatment, are critical to reducing stigma and improving the quality of life for men affected by ED.

**Keywords:** sexual dysfunction; stigma; comprehensive strategies; sexual health; awareness



## 1. Introduction

Erectile dysfunction (ED) can be defined as a sexual dysfunction characterized by the "inability to achieve and maintain sufficient penile erection for satisfactory sexual activity" (Sharma & Sharma, 2019). It is a multidimensional condition that may involve pathological changes in any component of the erectile response, with both endocrine and non-endocrine pathways—neurogenic, vasculogenic, and iatrogenic—playing an important role. It is also important to emphasize that ED is not exclusively age-related; on the contrary, an increasing number of younger men are affected by this condition (Yafi et al., 2016).

### 1.1 Etiology of erectile dysfunction

The etiology of ED is classified as organic (organic ED), psychogenic (psychogenic ED), or, most commonly, a combination of both (Allen et al., 2023). Among the psychological factors influencing ED, depression and anxiety are frequently highlighted. These factors play a crucial role even when organic causes are also present. Aging contributes significantly to ED, as cardiovascular diseases, hypertension, and other chronic conditions become more common with increasing age (Matsui et al., 2015). It is important to differentiate between psychological and organic causes in order to choose the most appropriate treatment approach for each individual.

#### 1.1.1 Vascular etiology

Vascular diseases, such as atherosclerosis and hypertension, are among the most common causes of ED. In atherosclerosis, the arteries narrow due to the accumulation of fatty deposits on the blood vessel walls, reducing blood flow to the penis— a crucial factor in achieving and maintaining an erection. Reduced blood flow can lead to insufficient oxygen supply to the penile tissue, resulting in impaired erectile function. Hypertension further damages the blood vessels, accelerates the progression of arteriosclerosis and worsens the condition. ED is often an early indicator of underlying vascular disease, underscoring the importance of early diagnosis and treatment of vascular disease to prevent ED. Timely detection and treatment of vascular disease is crucial to mitigate the long-term consequences on erectile function (Slowikowska-Hilczler et al., 2024).

#### 1.1.2 Neurological etiology

Neurological disorders, such as multiple sclerosis, Parkinson's disease, and spinal cord injuries, significantly impair erectile function. These diseases disrupt the transmission of signals between the central nervous system and the genital organs, leading to difficulties in achieving or maintaining an erection. Multiple sclerosis damages the myelin sheaths of nerve fibers, and slows down the transmission of nerve signals essential for initiating an erection. Parkinson's disease affects motor and autonomic functions, and has a similar effect on the erection process. Neuropathy associated with diabetes is another critical factor contributing to ED, as it damages the peripheral nerves that are important for normal erectile function (Saadi et al., 2024).

#### 1.1.3 Psychogenic etiology

Psychological factors such as stress, anxiety, depression, and relationship issues, contribute to the development of erectile dysfunction. Psychogenic ED is particularly common in younger men, where organic factors are less pronounced. Psychological stress associated with the onset of ED, as it activates the sympathetic nervous system, leading to vasoconstriction and reduced blood flow to the penis. Furthermore, chronic anxiety and depression decrease sexual desire and can affect the functioning of the hypothalamic-pituitary-gonadal axis, which further impairs erectile function. Fear of failure and shame due to previous incidents can create a vicious cycle, in which psychological pressure increases, leading to recurrent erectile issues (Pantazis et al., 2024).

#### 1.1.4 Impact of pornography

In recent years, research attention has increasingly focused on the impact of pornography on sexual health, particularly in relation to ED. Frequent use of internet pornography has been linked to various sexual dysfunctions, especially in younger men who have constant access to such content (Kizilkurt et al., 2023). One key finding is that frequent pornography use can lead to reduced sensitivity to typical sexual stimuli— resulting in desensitization. Men who frequently view pornography may develop a tolerance, meaning they require

increasingly extreme content to achieve the same level of arousal they experienced when they first consumed it (Kedman, 2021). In addition to physiological changes, pornography also has significant psychological consequences. Regular pornography use often creates unrealistic expectations of sexual intercourse, which can lead to increased anxiety about sexual performance, and subsequently, to psychogenic erectile dysfunction (Psychological Erectile Dysfunction: Mental Health and ED - Precise Men's Medical, 2021).

#### 1.1.5 Endocrinological etiology

Testosterone plays a crucial role in sexual desire, libido, and erection maintenance, as it influences the release of nitric oxide, which is necessary for the relaxation of smooth muscles in the penis and consequently increases blood flow (McCoskey & Vernon, 2024). Diabetes contributes to hormonal changes that can cause ED as it affects insulin levels, leading to insulin resistance and associated hormonal imbalances. This can result in reduced testosterone production and increased levels of stress hormones, such as cortisol, which have a negative impact on erectile function (Ilkhomovich, 2024).

#### 1.1.6 Traumatic factors

Injuries to the penis, pelvis, or spine can cause direct damage to nerves, blood vessels, and tissues, leading to ED. Injuries resulting from accidents, falls, or surgical procedures, such as prostatectomy, are common causes of traumatic ED. Surgical interventions, especially those involving prostate removal due to cancer, can damage nerves that are important for normal erectile function (Lounici et al., 2024). Such injuries can lead to persistent difficulties in achieving an erection if proper rehabilitation is not provided. Early diagnosis and treatment are crucial for the recovery of erectile function after traumatic injuries. This includes physical therapy, psychological support, and, if necessary, surgical reconstruction of the damaged tissues (Junejo et al., 2024).

#### 1.1.7 Factors related to the use of medications and other substances

The use of certain medications, such as antidepressants, antihypertensives, and antihistamines, is well-documented as a possible cause of the development or exacerbation of ED. Antidepressants, particularly selective serotonin reuptake inhibitors (SSRIs), are known to affect sexual function. These medications increase serotonin levels in the brain, which can reduce libido and cause difficulties in achieving or maintaining an erection, as they inhibit reflexes essential for erectile function. Additionally, antihypertensives, such as beta-blockers and diuretics, are associated with ED as they reduce blood flow to the penis by lowering blood pressure, which can lead to difficulties in achieving a sufficient erection (Andersen et al., 2024). In addition to medication use, certain substances—alcohol, tobacco, and recreational drugs—negatively affect sexual function and increase the risk of ED. Chronic alcohol consumption can lead to liver damage, which causes hormonal changes, including a decrease in testosterone levels, which has a direct impact on sexual function (Ilkhomovich, 2024).

#### 1.2. Social perception of masculinity and the impact of erectile function on an individual's psychological well-being

The erection of the penis has long been considered a symbol of male virility and sexual power. It is important to mention the traditional ideology of masculinity, which is a cultural construct that "defines a set of beliefs and expectations about how men should behave in a given time and culture" (Valsecch et al., 2023). This ideology emphasizes that a man must strive to achieve an appropriate status and dominance, while also being confident and dominant (Walther et al., 2023). The traditional ideology of masculinity also believes that masculinity is expressed through sexuality; therefore, a man must demonstrate his ability, performance, and dominance through sexual intercourse. This belief requires men to be heterosexual and hypersexual (Valsecch et al., 2023). From this perspective, an erection represents the foundation of masculinity, and sexual performance, providing satisfaction and procreation (Walther et al., 2023).

Despite societal progress, which has strongly encouraged open discussions on sexual life and health in recent years, topics related to ED are still avoided (Sheng, 2021). Sexual dysfunction is often surrounded by stigma, shame, and misconceptions. Unrealistic expectations regarding sexuality are frequently perpetuated in society, creating a standard that can make even men without dysfunctions feel inadequate (PosteryHealth, 2025). The mere

perception of problems - whether physiological or psychosocial—triggers a range of negative psychosocial reactions. The onset of ED is not only a physical problem but also an emotionally painful experience, involving fear, anxiety, and stress. The most significant (and often first) psychological reaction is the feeling of emasculation (i.e., loss of masculinity), which is closely related to the aspects rejected by the traditional ideology of masculinity (ED represents a contradictory aspect of this ideology) (Sheng, 2021). In addition to emasculation, the occurrence of ED affects a man's self-esteem and self-image. Due to the decline in self-esteem and self-confidence, many men isolate themselves socially when confronted with ED.

The social isolation, that often accompanies men with ED is due to the great fear of not being able to satisfy their partner, which leads to feelings of failure and fear of rejection or abandonment (Sheng, 2021). This results in decreased sexual activity (due to the emotional burdens mentioned), further destabilizing the partner dynamic, and causes feelings of undesirability, unattractiveness, and emotional frustration in the partner, which in turn can increase emotional distance and feelings of unlovedness (Huri et al., 2016; Sheng, 2021; Allen et al., 2023). Such emotional and intimate distance often leads to thoughts of infidelity or the impression that the partner is losing interest, which can jeopardize the long-term stability of the relationship and increase the likelihood of separation (Huri et al., 2016). Moreover, individuals with ED often experience a reduced quality of life, further burdening their daily lives (Allen et al., 2023). In addition to relationship issues, men with ED often face difficulties in friendships, as they believe that disclosing their problems will result in a lack of understanding and support. The lack of communication about ED further deepens feelings of loneliness and increases psychological stress. Open and effective communication is crucial to managing these issues, as the inability to express these experiences can heighten anxiety related to sexual function and have a negative impact on an individual's self-image and intimate relationships (Sheng, 2021).

### 1.3. Prevalence, risk factors, and the importance of awareness

A review of nine scientific studies on the prevalence of ED reveals significant trends related to age, risk factors, and awareness of this issue. Research shows that the prevalence of ED increases significantly with age, which is confirmed by several studies. For instance, Saramies et al. (2022) found in a prospective cross-sectional study involving 189 men that ED was present in 100% of men over the age of 75 (at least in a mild form). Similar findings are reported by Çayan et al. (2017)—the prevalence of ED was 17% in men aged 40 to 49, years and 82.9% in men aged 70 years and older. A study conducted in China by Zhang et al. (2017) which included 5210 people over the age of 40, also confirms that the prevalence of ED increases with age.

Other studies indicate that ED occurs more frequently in men who have risk factors such as an unhealthy lifestyle, obesity, smoking, and alcohol consumption. In a study by Li et al. (2022), which included 12,490 men in the United Kingdom, a strong correlation was found between the incidence of ED and unhealthy lifestyle habits. Hallanzy et al. (2019) found in a sample of 2,565 men that, the prevalence of ED (25.2%) increased with the number of risk factors, with 48.8% of individuals reporting severe symptoms. A large majority of studies also emphasize the importance of awareness and education for the prevention and early treatment of ED. Herkommer et al. (2023) highlight the significance of risk factor education as a fundamental step in reducing the prevalence of ED. Similarly, Rezali et al. (2023) in a study conducted in Malaysia stress the need for comprehensive sexual education to improve the understanding and treatment of ED. Oyelade et al. (2016) emphasized the importance of raising awareness among healthcare providers about the impact of an individual's sexual history on diagnosis and treatment planning. The common focus of these studies suggests that comprehensive awareness strategies are crucial for reducing the risk of ED and improving access to appropriate care.

Strategies to enhance awareness and access to ED treatment are essential, as they enable early identification of issues, encourage preventive measures, and improve access to effective treatment options, which significantly contribute to the quality of life for men with ED. Raising awareness about ED is important for reducing stigma and discrimination and encouraging men to seek appropriate treatment. Educational programs that promote an

open approach to sexual health have shown that men are more willing to seek help when they have access to reliable information (Moazin et al., 2024).

## 2. Strategies for improving awareness and access to erectile dysfunction treatment

ED is a health condition that affects many men worldwide, but is often misunderstood and stigmatized. Many men, and even some healthcare providers, fail to recognize the physiological causes of ED, such as cardiovascular diseases or endocrine disorders, which can lead to delays in seeking help and the worsening of underlying health problems (Bolash & Mekhail, 2024).

Therefore, raising awareness of ED is crucial to reduce stigma and discrimination and encourage men to seek appropriate treatment (Moazin et al., 2024). It is also important to highlight the challenges of reaching all populations, especially those who are more socially and culturally isolated. For this reason, it is essential to develop a multi-layered approach that not only reduces stigma but also increases the accessibility to care and provides effective support for men affected by ED. This approach includes public health campaigns, the proactive inclusion of sexual health in routine check-ups, culturally adapted strategies, education, counseling, and the use of modern technologies such as telemedicine.

### 2.1 . Public health campaigns with a clear message

One of the most important strategies for reducing the stigma associated with ED is public health campaigns aimed at educating the public about the nature of this condition. Awareness and education help to reduce stigma and increase understanding of ED (Imran et al., 2023). Public health campaigns, that disseminate information through various media, can reach a wider population and encourage open dialogue about sexual health. Such campaigns not only reduce the stigma associated with ED but also encourage men to seek professional help without fear of shame or judgment (Tavakoli et al., 2023).

These campaigns must clearly highlight the medical background of ED and encourage men to seek help without fear of condemnation. It is important for these campaigns to emphasize that ED is not just a result of physiological changes, but can also be linked to various other factors, including psychological issues, lifestyle habits, and chronic diseases. Campaigns should dispel the myth that ED is a lack of masculinity and present it as a manageable health problem that can be treated if recognized early and addressed properly. Including personal stories of men who have successfully overcome ED can reduce feelings of loneliness and increase social support within this population, which is crucial to reducing feelings of isolation (Imran et al., 2023).

### 2.2 Integration of sexual health discussions into routine health check-ups

A better understanding of the causes, symptoms, and treatment options of ED enables men to actively participate in their treatment and improve their chances of a successful recovery. Additionally, training healthcare providers to recognize and effectively manage ED is essential for improving the quality of care patients receive (Esposito et al., 2008). Healthcare providers, particularly primary care physicians, play a key role in the early detection and treatment of ED. Physicians and nurses need to take a proactive role in incorporating sexual health issues into routine health check-ups. Many men do not address erectile problems on their own because of shame or fear of stigmatization themselves. Therefore, it is important for healthcare providers to actively initiate an open dialogue about sexual health, using a sensitive approach that allows patients to feel safe and ready to share their concerns. Moreover, healthcare providers must have access to appropriate training and resources to fully recognize and treat ED, as this condition often involves both physiological and psychological factors. It is crucial to ensure that healthcare providers are well-equipped with the knowledge to provide holistic care for this issue, which can reflect broader aspects of the patient's health (Esposito et al., 2008).

### 2.3. Culturally adapted campaigns

Cultural norms and values have a significant impact on the perception of ED and the willingness of men to talk about it or seek help. In some cultural environments, ED is associated with a loss of masculinity, leading to social stigma and delays in seeking help. A lack

of understanding of cultural specificities can lead to inadequate approaches to the treatment and support of men suffering from ED. Therefore, it is crucial for healthcare institutions and professionals to consider these cultural and social factors when addressing ED (Jamali, 2024). Awareness of the importance of a culturally sensitive approach to this issue is key to successfully reducing the stigma associated with ED in various communities. ED awareness campaigns need to be tailored to specific cultural norms and expectations. In cultures where sexual health issues are often considered taboo, it is especially important to involve local leaders, influential individuals, and healthcare professionals who are respected in the community. Such an approach can help destigmatize ED, as men seeking help in these communities often expect support and understanding from their cultural and religious leaders. Involving these influential figures in awareness-raising efforts is essential for encouraging men to seek professional help and recognizing ED as a manageable health issue that should not be a source of shame (Jamali, 2024)

#### *2.4. Improving access to education and counseling within the healthcare system*

The treatment of ED requires a holistic approach that includes both patient education and healthcare providers training. The healthcare system must actively promote education about ED, not only to patients but also to healthcare professionals who often have the first contact with men facing this condition. Nurses, physicians, and other healthcare providers must be adequately trained to recognize the symptoms of ED, make a diagnosis and offer treatment, while creating a supportive environment where men feel comfortable discussing their issues. It is essential that healthcare professionals regularly update their knowledge of new research and therapies related to ED, so they can offer the best possible care to patients. Continuous improvement of knowledge and training of healthcare providers in primary healthcare plays a crucial role in the early detection and effective treatment of ED (Moazin et al., 2024).

#### *2.5. Use of telemedicine and digital solutions*

For many men, particularly in smaller communities or rural areas, feelings of shame and lack of privacy can be a significant barrier to seeking personalized medical care. Telemedicine offers an innovative solution to this problem by enabling anonymous and secure communication between patients and healthcare professionals. Through telemedicine consultations, men can receive all necessary information about ED, discuss potential treatment options, and decide on next steps without the need for an in-person visit. Additionally, digital platforms that offer educational content about ED can help raise awareness and provide access to verified information. This enables men to easily access expert advice and information, allowing them to make more informed decisions about their health without having to leave the house. Therefore, telemedicine not only reduces stigma but also facilitates access to treatment and counseling, especially in situations where geographical or cultural limitations would otherwise hinder access (Imran et al., 2023).

Reducing stigma and improving access to ED treatment requires a multi-faceted approach that includes public health campaigns, the integration of sexual health into routine check-ups, culturally adapted strategies, broader education for healthcare providers, and the use of modern technologies such as telemedicine. Only through openness, support, and proper training of healthcare professionals can stigma be reduced and a better quality of life be provided for men with ED. It is crucial to recognize ED as a manageable health condition that deserves the same attention and treatment as other health problems.

## **5. Conclusion**

The stigma associated with ED remains a significant barrier to accessing treatment. Comprehensive strategies, including awareness campaigns, the integration of sexual health into routine medical check-ups, and culturally adapted approaches, are crucial for reducing stigmatization and increasing access to ED care. Training healthcare providers to approach these issues in a non-judgmental way is also important to enable open communication between patients and physicians. Further research is needed to investigate the long-term effects of these strategies and their potential adaptation to specific cultural contexts.

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Reflection

# On the Music of the 12<sup>th</sup> Socratic Lectures. Z-STEAM and Nanostructurome activities

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## Abstract:

We describe and reflect on the cultural events connected to 12th Socratic Lectures. The satellite events included, in the chronological order: the performance of Lara Oprešnik at the Musikverein, Vienna within the New year concert on January 8, 2025, the Z-STEAM social networking event with the concert on January 11, 2025 at the Julij Betteto Hall, Kazina, Academy of Music, University of Ljubljana, and the organ recital of Roberta Schmid from Napoli, Italy, at the Church of Assumption, Tromostovje. Socratic Lectures continue as international events promoting science and arts.

**Keywords:** Baroque opera; Roman history; Venice carnival; Nero Claudius Caesar Augustus Germanicus; Sabina Poppaea the Younger; Commedia dell'Arte



### 1. The concept of Socratic Lectures

According to Plato, a man named Socrates and his pupils assembled in a place near to the sea. They were discussing things that interested them in a pleasant atmosphere. Socrates stated and the pupils responded as to agree or disagree. Then they asked questions. It required little knowledge (in the form of data) from the pupils but a lot of curiosity and reasoning. The dialogues were respectful and polite and created a room of gentleness. It only took acknowledging and liking it to enter this room, which seemed simple and easy. In contrast to sophistic teaching, there was no formal scheme and no exchange of money. However, Plato's interpretation of the significance of money concerns not just teaching, but in fact all human activities (Schindler, 2009). Socratic Lectures aim at reaching the spirit of interest in the matter, common well being, gentleness and politeness above all other reasons. In particular, it is a point where excellent scientists share their experience with the students and the students contribute their dedication. Also, it is a point of meeting of different fields from science and arts to inspire creation of new ideas. The team of Socratic lectures is constituted of those who have donated as the participants of the activities of the Socratic Lectures.

### 2. Z-STEAM activities and integration of science and arts

STEAM (Science, Technology, Engineering, Arts, Mathematics) Education is an approach to learning that uses Science, Technology, Engineering, Arts, and Mathematics as access points for guiding student inquiry, dialogue, and critical thinking. While this approach has been acknowledged at the primary and high school level, it has only recently been introduced to higher education (Carter et al., 2021). Socratic Lectures have early integrated visual arts and music with science and are open for other forms of art and achievements aiming at life-friendly world and excellence. Formal collaboration has been established between nine faculties of the University of Ljubljana and the Academy of Music within the project Nanostrukturoume financed by the Slovenian Research Agency in collaboration with the University of Ljubljana. Participants from the Academy of Music contributed lectures at the symposium devoted to baroque music, in particular the work of Claudio Monteverdi, and performed at the concert selected pieces from Monteverdi's opera Coronation of Poppaea. The partners of the project Nanostrukturoume will support the production of the opera with the premiere set in July 2025.

### 3. Music events of the 12-th Socratic Lectures

The satellite events included, in the chronological order: the performance of Lara Oprešnik at the Musikverein, Vienna within the New year concert on January 8, 2025, the Z-STEAM social networking event with the concert on January 11, 2025 at the Julij Betteto Hall, Kazina, Academy of Music, University of Ljubljana, and the organ recital of Roberta Schmid from Napoli, Italy, at the Church of Assumption, Tromostovje. Socratic Lectures continue as international events promoting science and arts.

#### 3.1. Lara Oprešnik and her performance at the Musikverein, Vienna, within the New year concert

Lara Oprešnik started playing piano at the age of seven in the class of Professor Monika Vehovec and violin in the class of Professor Igor Ulokin. Later she studied with Professor Jožica Grebenšek and Professor Jernej Grebenšek. She graduated from the Academy of Music in Ljubljana, Slovenia, under Professor Hinko Haas and from the University of Music and Performing Arts in Graz, Austria, under Professor Milana Černjavška. In 2023 she received her Master's degree from the Academy of Music in Ljubljana, in the class of Professor Ruben Dalibaltayan. She studied with Laszlo Baranyay, Jasminka Stančul, Lovro Pogorelić, Rita Kinka, Oleg Maršev, Natasha Kudritska and Julia Gubajdulina. During her studies she received the Prešeren Student Prize of the University of Ljubljana. She has performed as a soloist with orchestras at home and abroad. In 2024, she recorded Saint-Saëns' chamber work Animal Carnival and a longer programme of her own compositions, approximately one hour long, as part of the From the Pen of Slovenian Composers series. She has given solo recitals at numerous national and international festivals (Vienna, Trieste, Tenerife, Lamporecchio, Ljubljana, Gorizia, Rome, Citta di Cervignano, Belgrade, etc.). On 8 January 2025, she performed with the Vienna City Orchestra at the gala New Year's



Concert at the Vienna Musikverein as the first prize-winner of the Vienna New Year's Concert, an international music competition.

Miha Červ who attended the concert at the Musikverein (**Figure 1**), reflected on the performance of Lara Oprešnik: "I wore the best dress I own - my wedding dress. I didn't want to go to the Musikverein dressed casually, because that's where the fine gentlemen usually gather. But it turned out that my fears were unnecessary, because the assembled company was very varied in the formality of their dress, and so I stood out neither as too well dressed nor as too poorly dressed. The concert was intended to showcase the soloists - the winners of the competition, accompanied by the Wiener Stadtorchester (Vienna City Orchestra) under the baton of Christian Schulz. Almost exactly at eight o'clock, the orchestra members came on stage and tuned up. The first part of the concert was introduced by the orchestra itself with a piece by Handel, and then the soloists took the stage for the following pieces; viola, two opera singers and oboe. After the interval, I returned to the hall, where a large concert Steinway had just appeared on stage. My neighbour, with whom I had chatted earlier, whispered to me: 'Jetzt kommt Ihre Favoritin!'. And after the orchestra had tuned up again, Lara Oprešnik started. She played the first movement of Scriabin's concerto for piano and orchestra in F sharp minor. The performance was sovereign and dramatic. The orchestra and Lara were well matched and the performance of the piece was without any perceivable mistakes for me. Lara earned a roaring applause and it was interesting to see her bow to the audience laughing after such a serious piece. This was followed by a piece for which the conductor changed and the winner of the conductor category took over the orchestra, and then a reappearance of the opera singer. The concert ended with a piece written by Christian Schulz himself. At the end of the music, the conductor presented the prizes to the competitors. I may be a little biased, but I would say that Lara received a little more applause than the other prize winners. At the end I had the opportunity to congratulate the pianist briefly and then I returned home under the good impression of the musical evening."



**Figure 1.** Left: performers after the concert at Musikverein. Left: Lara Oprešnik and Christian Schulz.

### 3.2. Social event at the Betteto hall with concert of classical and contemporary music and poetry

The concert(**Figures 2-5**) had three parts , the first part was devoted to classical music, followed by an intermezzo of poetry by Ifigenija Simonović and Slovene choral music. The



third part was devoted to contemporary music, with emphasis on original compositions integrating science.

**Part I: Classical music**

- C. Franck: Petit offertoire. Organ: Yelena Istileulova
- C. Monteverdi/G.F. Busenello: Prologue from opera Coronation of Poppaea. Sopranos: Fortuna: Ronja Prapotnik, Virtu: Eva Kokot, Amore: Brina Vukovič, Harpsichord: E. Mihajlovič, Organ positive: B. Rezič
- A. Aljabjev A: Nightingale. Flute: Anita Prelovšek, Piano: Elena Startseva Somun
- S. Rachmaninov: Vocalise. Violin: Vasilij Meljnikov, Piano: Lara Oprešnik
- C. Monteverdi/G.F. Busenello: Regina disprezzata from opera Coronation of Poppaea. Soprano: Ottavia: Alessandra Tessaro, Harpsichord: E. Mihajlovič, Organ positive: B. Rezič
- S. Rachmaninov: Etude Tableaux Op 33 No 3. Piano: Lara Oprešnik
- G. Brun: Romance. Flute: Anita Prelovšek, Piano: Elena Startseva Somun
- C. Monteverdi/G.F. Busenello: Duetto Demigella and Valetto from opera Coronation of Poppaea. Sopranos: Demigella: Nives Hadžić, Valetto: Eva Kokot, Harpsichord: E. Mihajlovič, Organ positive: B. Rezič
- W.A. Mozart: Alla turca from Sonate in A major. Piano: Denis Luin
- F. Chopin: Polonaise G sharp minor. Piano: Matic Bogataj

**Part II:**

- G. Ipavec/A. Čopi - Simon Gregorčič: Mountain flower: Chorus Studenec  
Poetry by Ifigenia Simonovič
- H. Lavrenčič/Anonymus: The coque has sung: Chorus Studenec
- E. Adamič/O. Župančič: Evening song: Chorus Studenec

**Part III: Contemporary music**

- A. von Sultanova/Titus Lucrecius: De rerum natura. Piano, Voice: Aleona von Sultanova S Kralj; Topology. Piano: Samo Kralj
- H. Mancini: Pink Panther. Trombone: Emil Somun, Piano: Elena Startseva Somun
- A. von Sultanova/A. von Sultanova: For Samuel Gmelin - water, water. Piano, Voice: Aleona von Sultanova
- J. Rae: Sonatine (Aquarelle, Notturme, Firedance). Flute: Anita Prelovšek, Piano: Elena Startseva Somun
- A. Schnittke: Suite old style. Violin: B. Brezavšček, Piano: E. Startseva Somun
- L. Oprešnik: Fugue. Piano: Lara Oprešnik
- B. Kobal: Dic verbo. Soprano: Kaya Tokuhisa, Organ: Jana Jamšek
- D. Zupanič Turkovič: Cantique de Baruch Spinoza from Mass in E minor. Organ: A. von Sultanova, Percussion: Bojan Ilievski, Piano: Lara Oprešnik, Flute: Anita Prelovšek

**Egon Mihajlovič** studied harpsichord and early keyboard instruments at the Frankfurt Conservatory of Music and Performing Arts, where he also completed his postgraduate studies. Since 1990 he has performed as harpsichordist, conductor and artistic director of baroque opera and sacred baroque music at major concert venues and festivals in Europe and the United States. He has taught at music art colleges and music academies in Wurzburg, Nuremberg, Cetinje and Pesaro. He is currently Professor of Harpsichord and Head of the Department of Early Music and Head of the Chair at the Academy of Music of the University of Ljubljana. In 2013 he was awarded a golden plaque of the University of Ljubljana for his outstanding contribution to the development of scientific, pedagogical or artistic endeavours.

**Anita Prelovšek** studied flute with Professor Anđela Blažeka in Ljubljana, Professor Luisa Sello in Udine and Trieste, Professor Karolina Šantl-Zupan in Ljubljana and Professor Gladys Bouchet at the Conservatoire National de Région in Rennes, France. She graduated at the Giuseppe Tartini Conservatory of Music in Bologna and specialised at the "Istituto Superiore di Studi Musicali O.Vecchi-A.Tonelli" in Modena, where her professors were Gabriele Betti, Michele Marasco and Andrea Oliva. She performs as a soloist and in various chamber ensembles in concerts at home and abroad.



**Figure 2.** Upper left: Matic Bogataj, upper right: Anita Prelovšek, lower left: Denis Luin, lower right: Lara Oprešnik, all at the concert of the 12.th Socratic lectures. Photo: Drago Videmšek.

**Ronja Prapotnik, Eva Kokot, Brina Vukovič, Alessandra Tessaro and Nives Hadžič** study singing at the Academy of Music, University of Ljubljana.

**Elena Startseva Somun** studied piano at the Pyotr Ilyich Tchaikovsky Moscow Conservatory of Music and did postgraduate studies in chamber music at the Far Eastern Academy of Arts and Music in Vladivostok. She performs as a soloist and in chamber ensembles at home and abroad. She has taught at the Sergei Prokofiev Music School in Vladivostok, the Jilin Academy of Music in Changchun, China and the Idrija Music School.

**Violinist Vasily Melynikov** studied at the Minsk Academy of Music with Olga Parkomenko and at the Moscow Conservatoire of Pyotr Ilyich Tchaikovsky with Professors Bezrodny and Belenka. In 1997 he won first prize at the Vienna Modern Masters International Competition in Vienna. He has been a member of the Belarusian State Chamber Orchestra, the Camerata Slovenica String Quartet, the Ljubljana Piano Trio and the Zagreb Soloists Orchestra. From 2004 to 2010 he was concertmaster of the RTV Slovenia Symphony Orchestra. He has taught at the Ljubljana Conservatory of Music and Ballet and at the Academy of Music, where he was head of the string department and mentor of the Academy of Music Symphony Orchestra. Vasilij Meljnikov is the recipient of the Betetti Prize (2005) for artistic achievement in the field of creative music and for his efforts to spread musical culture.

**Emil Somun** is studying trombone in the class of prof. Matija Mlakar. He performed at the Z-STEAM events connected to the Socratic lectures in Ljubljana and Naples since 2022.

**Ifigenija Simonovič** is writing poetry, essays, book reviews, translating, and painting various objects for more than fifty years. She is the author of ten poetry collections, three books of essays, three books for children, and is also known as a publisher of eight books of poetry by Vitomil Zupan. In 2009, she received the Rožanec award. Between 2017 and 2021, she was the president of the Slovenian PEN Center. Her poetry is often dark, but love persistently shines through the cracks that are drawn on her path by real-time.

**Chorus Studenec Pivka**, led by **Irena Rep**, is composed of experienced singers. In its 22 seasons, various genres were performed but the singers prefer to sing arrangements of folk



songs. They have around 30 concerts annually, home and abroad, most importantly, the visits to Slovenian societies throughout Europe.



**Figure 3.** Upper left: Ronja Praprotnik, Eva Kokot and Brina Vuković performing Prologue from Monteverdi's Coronation of Poppaea, upper right: Jana Jamšek, Barbara Kopal and Kaya Tokuhisa performing Sic verbo by Barbara Kopal. Lower left: Ifigenija Simonović reading her poetry, lower right: chorus Studenec performing Slovene choral. Photo: Drago Videmšek.

**Samo Kralj** is playing piano in PhD Big Bang Band since 2007. The band consists of 7 researchers from all over Slovenia and they have on average 3 performances per year.

**Violinist Branko Brezavšček** graduated from the Academy of Music in Ljubljana in the class of Prof. Dejan Bravničar. He was a member of the Slovenian Philharmonic Orchestra, concertmaster and soloist of the chamber ensemble Carnium, member and soloist of the Slovenian Chamber Ensemble under the direction of conductor Anton Nanut, first violinist of the Muzina Quartet, concerto soloist accompanied by piano, guitar and organ. He performed in Slovenia, Austria, Italy, Germany and USA. He recorded for RTV Slovenia and Radio Trieste.

**Kaya Tokuhisa** studied classical singing with professors Tatjana Vasle, Maria Cristina Kiehr, Ulrich Messthaler, Harry van der Kamp, Snežana Nena Brzaković, Marianna Váradi, Thomas Heyer, Edita Garčević Koželj and Urška Arlič Gololičič, and chanson with professor Žare Prinčič. She received Master's degree in Theatre singing in 2023. Together with the Hungarian pianist Beata Barcza and in ensembles she performs in concerts home and abroad. She performed at the Slovenian Philharmonic in the honour of the visit by His Imperial Highnesses prince and princess Akishino in 2013. With pianist Rok Lopatič and accordionist Žiga Vehovec, they formed a band called Café Noisette.

**Barbara Kopal** attended musical school in Idrija. She mainly composes music and writes lyrics for chansons and songs. Her works were performed in public. The composition "It snows" was performed in Cankarjev dom and Slovenian Philharmonic, Ljubljana where it was warmly received. She participated at festivals Spevslam and Slovenian chanson and



at numerous cultural events. This is her first presentation at the Z-STEAM event satellite to the 12th Socratic Lectures symposium.

**Jana Jamšek** studied piano with Prof. Irma Hladnik and later organ at the Organ School in Ljubljana with Prof. Gregor Klančič. She has appeared as a soloist in interpretations of piano works by J.S. Bach, C. Saint-Saens and J. Brahms, in chamber ensembles with instrumentalists and singers, and as an accompanist for classical ballet. She has been participating in the Socratic Lectures since 2008. As an organist, she performs in concerts of the Organ School and in services at the Trnovo Church.

Aleona von Sultanova received a diploma at the Music College Tchaikovsky in Kazakstan. She studied saxophone, and is presently studying organ at the Theological Faculty, University of Ljubljana. She is composing and performing poems (voice and piano). For her compositions, she received international awards at the Festival of Russian Romances (Škofja Loka) and Festival of Children Songs Veseli veter (Novo Mesto). She performed at the International UNESCO Sharq Taronalari festival in Samarkand, Uzbekistan. In 2024 she received an award from South Arts and Health Envoy and 2 Platinum and 2 Gold prizes at the Max Bruch International Music Competition. She is the ambassador of ESTEAM (Entrepreneurship + STEAM: Science, Technology, Engineering, Arts and Mathematics) Program of the European Union to empower girls to pursue a STEM career and an inventor of SMS (Stories based on Music about Scientists) method. She performed at satellite events to the Socratic lectures and Z-STEAM events in Ljubljana and Naples, Italy. In 2024, she had a concert at the Academy Enrico Caruso, Naples, Italy.



**Figure 4.** Upper left: Alessandra Tessaro, Ronja Praprotnik, Eva Kokot, Brina Vuković and Nives Hadžič, upper right: Anna Romolo and Veronika Kralj-Iglič with Pupa, lower right: Jelena Danilović Luković, Ani Barbulova, Anna Romolo, Aleš Iglič and Veronika Kralj-Iglič. At the back: Lara Oprešnik. Lower right: from the left: Elena Startseva Somun, Yelena Istileulova, Lara Oprešnik, Anita Prelovšek, Veronika Kralj-Iglič and Bojan Ilievski. Photo: Drago Videmšek.



**Davorin Zupanič Turković's** opus comprises instrumental and vocal compositions and adaptations that have been performed at the satellite events of the Socratic Lectures symposia, Z-STEAM events of scientific meetings and at the openings of cultural events in Ljubljana, Piran, Novo mesto, Šentrupert, Belgrade and Naples. In 2021, the quintet Spectrum performed the SARS ORF1 composition written especially for this event - at the opening of the 45th FEBS (Federation of European Biochemical Societies) Congress in Ljubljana. The Mass in E minor with Kyrie, Credo, Alleluia, Agnus Dei and Cantique de Baruch Spinoza was performed for the first time in 2018 at the Church of the Assumption on Tromostovje.



**Figure 5.** Up: Yelena Istileulova. Lower left: Branko Brezavšček, lower right: Lara Oprešnik and Vasily Meljnikov. Photo: Drago Videmšek.



### 3.3. *Organ recital of Roberta Schmid*

The program of the recital:

D. Buxtehude: Passacaglia in D minor BuxWV 161

J. S. Bach: Choral Das alte Jahr vergangen ist BWV 614

J. S. Bach: Choral Erbarme dich mein Gott BWV 721

A.G. Ritter: Sonate no. 2 in E minor op.19

G. Mushel: Toccata

J. Rheinberger: Passacaglia

L. Vierne: Carillon de Westminster

Roberta Schmid has contributed significantly to the Z-STEAM activities in Ljubljana and in Naples, Italy since 2019. In 2024, she participated at the concert that took place in Villino Manina organized by the University of Ljubljana, Faculty of Electrical Engineering and Faculty of Health Sciences, and Consiglio Nazionale delle Ricerche, Napoli, Italy. At that concert, she performed on the piano a Slovene song entitled "Marinki" recently composed by Davorin Zupanič Turković, thereby promoting contemporary Slovene music internationally. Roberta Schmid took her diploma in Organ and Organ Composition at the Musical Conservatoire "S. Pietro a Maiella" in Naples with the mentorship of prof. A.M.Robilotta and then improved her technique with maestros of international standing such as L. Ghielmi, E. Kooiman, M. Radulescu, D. Roth, K. Schnorr, L. F. Tagliavini, M. Torrent, P. Westerbrinck, W. Zerer, specializing her executive practice in different organ repertoires. After getting through a national examination she attended a three year course in Professional training as an Organist at the Musical Academy in Pistoia and took a diploma in "Italian and German Organ Music Interpretation". She attended a three year course in Gregorian Chant at the International Study Centre of Gregorian Chant in Cremona. She also took diplomas in Piano and in Musical Didactics, and, lately, the second level degree specializing in Organ interpretation and composition, recently introduced in Italian Music Conservatoires. She has a very busy professional life performing at concerts as a soloist and in chamber formations. As a soloist, she has been invited to take part in numerous international organ festivals - in Europe and in Mexico - and she has always been highly acclaimed by public and critics alike. She also performs for: Quarto Festival Internacional de Organo de Zamora Michoacán (Mexico), Primer Ciclo Internacional de Conciertos en el Organo Barroco de la Parroquia de Santiago de Querétaro (Mexico), International Organ Summer Festival in Rome, XXVIII Festival Internazionale di Noale, Rassegna Antichi organi di Piacenza, Associazione Alessandro Scarlatti di Napoli, Associazione Studi Mezzogiorno, Associazione Ricercare, Accademia Organistica Campana, Rassegna organistica internazionale di Avezzano, Rassegna organistica di Pescara, Settembre Organistico Fabianese, Rassegna organistica veneta "Musica nell'Agordino", Rassegna "Musicalia" in Pavia, Festival Organistico Internazionale Città di Senigallia, Rassegna Organistica di Fiemme (Trento), Rassegna organistica della Svizzera italiana and for the International festivals performed at Notre Dame de Compassion in Paris, Merano Cathedral, Amalfi Cathedral, S. Vitale's Basilica in Ravenna, S. Ambrogio's Basilica in Milan and in Mexico City. She has recorded a Cd of Bach and pre-Bach music sponsored by the Goethe-Institut Italien. She is Artistic Director of the Festival "Musica intorno all'organo" at the St. Maria della Rotonda Church in Naples with the contralto Daniela Del Monaco. She was Artistic Director of the Concorso Organistico Nazionale Città di Napoli "11 Fiori del Melarancio". She taught "Organ and Organ composition" at F. Vittadini Civic Institute in Pavia and Organ at High School Alfano I in Salerno and at High School Palizzi in Naples. She is the official organist of Mascioni mechanical organ at St. Maria della Rotonda Church and at Santa Chiara's Basilica in Naples.





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# Analyzing Bending Energy in the Nuclear Pore Complex from a Point Cloud Representation

Grad Blaž<sup>1</sup>, Kralj-Iglič Veronika<sup>2</sup>

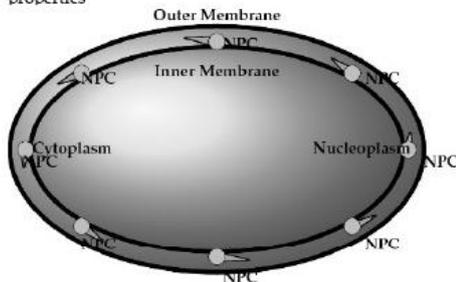
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## ABSTRACT

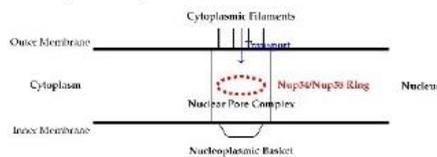
The Nup54/Nup58 complex, a critical component of the Nuclear Pore Complex (NPC), regulates the flexibility and diameter of the NPC's transport channel, adapting to varying cargo sizes. Using structural data from PDB entry 3T98, which represents a segment of the Nup54/Nup58 ring, we analyze its bending energy and flexibility through computational modeling.

Applying the Helfrich model in a discrete form, we compute the bending energy by deriving curvatures and surface area elements from the point cloud representation of 3T98. Our analysis of this dynamic NPC component provides insights into the energetic properties.



Nuclear Envelope with Nuclear Pore Complexes (NPCs)

**Figure 1.** Schematic representation of the nuclear envelope showing the inner and outer membranes and nuclear pore complexes (NPCs).



**Figure 2.** Schematic representation of the nuclear pore complex (NPC), highlighting key structural components such as the Nup54/Nup58 ring, cytoplasmic filaments, and nucleoplasmic basket.

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## METHODOLOGY FOR DATA PROCESSING

### Dataset Acquisition:

Structural data for the Nup54/Nup58 complex was obtained from PDB entry 3T98.

This dataset represents a segment of the Nup54/Nup58 ring, capturing interacting domains between these nucleoporins.

### Surface Representation:

The .cif file was processed in Chimera, where the molecular surface was visualized using the hydrophobic surface preset and the protein preset.

The structure was exported as a .obj file, capturing the molecular surface geometry.

### Point Cloud Generation:

The .obj file was imported into MeshLab, which exported the structure as a .xyz file (point cloud format containing coordinates and normals).

### Point Cloud Preparation:

The .xyz file was loaded into CloudCompare, where the data was further refined and converted into the .pcd format (Point Cloud Data) for computational analysis.

## METHODOLOGY FOR COMPUTATIONAL AND PHYSICAL ANALYSIS

### Curvature and Area Computation:

Using the Point Cloud Library (PCL), the following properties were computed for each point in the cloud:

Mean curvature ( $H$ ): Average surface bending. Gaussian curvature ( $K$ ): intrinsic curvature based on the product of the principal curvatures.

Area elements ( $\Delta A$ ): Local surface area around each point.

### Bending Energy Calculation:

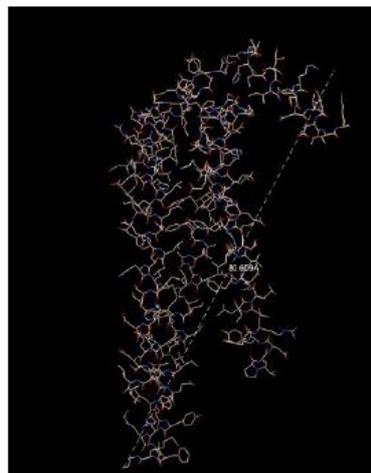
The bending energy was estimated using the Helfrich model in its discrete form:

$$E = \sum_{i=1}^N [k/2 (2H_i - C_0)^2 + k_c K_i] \Delta A_i$$

### Algorithmic application:

Algorithms are applied on the Point Cloud dataset to compute curvatures ( $H$  and  $K$ ), local surface area elements ( $\Delta A$ ). Filtering the area elements ( $\Delta A$ ) and finally computing the bending energy.

## RESULTS



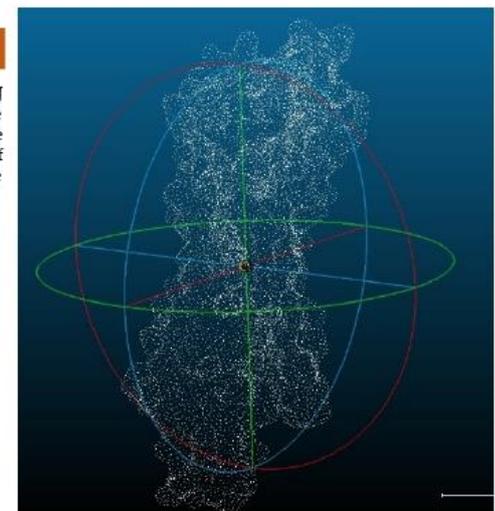
**Figure 3.** The Nup54/Nup58 structure open in Chimera using a protein display where the diagonal distance was measured to be about 80,659 Å or about 8.0659 nm.



**Figure 4.** The Nup54/Nup58 structure open in Meshlab showing the hydrophobicity surface geometry.

## COMPUTATIONAL RESULTS

The calculated bending energy of  $1.15363 \times 10^{20}$  J aligned with the expected range for flexible protein structure. This suggests that the Nup54/Nup58 ring segment exhibits a degree of flexibility comparable to other biomolecule complexes.



**Figure 5.** The Nup54/Nup58 structure open in CloudCompare, displayed in point clouds, showing the dataset ready for further computational analysis.

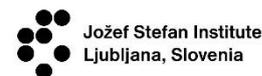


# Physical Approach to the Application of Numerical Methods in Analysing Race Car Dynamics and Performance on a Racetrack Using GPS Data

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## OUTLINE

The dynamics of racing cars and their modifications play an important role in motorsport performance. The ability to analyse and optimise the behaviour of the vehicle and driver on the race track is crucial for improving lap times and overall efficiency. In this project, numerical methods are used to analyse and improve the dynamics of the race car and the driver's performance using GPS data collected with the Race Chrono app. The main objectives were to gain insights into cornering behaviour, acceleration and braking. The main objective of this research was to develop a systematic approach to process real-world data collected after a racing event to extract critical parameters that affect vehicle performance. By integrating computational tools with experimental measurements, the study aimed to provide actionable insights to improve performance.



Figure 1: Vauxhall corsa VXR on a racetrack (Photo: R. Jošt).

Race cars (Figure 1) are modified vehicles specially designed to operate under extreme conditions on the race track, e.g. high temperatures, G-forces and abnormal material consumption. A high performance racing car is complemented by an experienced racing driver. Professional drivers seek to maximize performance by utilizing both the vehicle's capabilities and strategic driving techniques. Analytical studies such as this provide valuable insight into areas where lap times can be improved to increase the overall synergy between the vehicle and the driver.



Figure 2: Experimental setup in a Porsche GT3RS with Race Chrono lap time (Photo: <https://racechrono.com/#about>).



Figure 3: View of the top speed achieved (Photo: Race Chrono application).

In this investigation, we imported GPS data from the application (Figure 3), which contained key information such as latitude, longitude, altitude, vehicle speed and timestamp. This data provided an important starting point for our analysis as it contained real measurements that could be processed using numerical methods. Using this data, we were able to examine the driver's behaviour and identify areas where performance could be improved. Optimization strategies investigated in the study included braking earlier, refining the racing line and evaluating the impact of improved vehicle acceleration. These optimizations are crucial to improving vehicle and driver performance, ultimately leading to better lap times and more efficient use of the track.

## EXPERIMENTAL METHODS

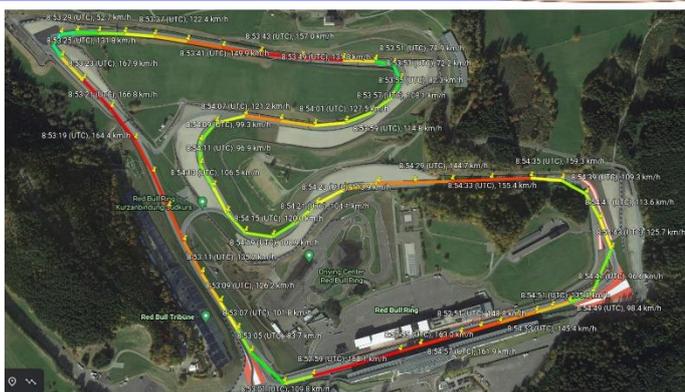


Figure 4: View of the merged data with timestamps and speed information (Photo: V. Nemeč).

### Data collection:

The GPS data was recorded during several laps on a specific race track using the GPS module built into the smartphone. The phone recorded the position, speed, geodetic coordinates and timestamp of the vehicle. This data served as the basis for analysing the vehicle's performance and trajectory.

### Preprocessing of the data:

The raw GPS data was filtered to remove noise and anomalies. To simplify the analysis, the GPS coordinates were converted into a Cartesian coordinate system. This step enabled a more efficient calculation of speed, acceleration and other parameters.

### Numerical methods:

The speed and distance travelled were calculated using finite difference methods that approximate the rate of change of position over time. Interpolation techniques were used to reconstruct the optimal racing line and generate a continuous trajectory. Numerical optimization algorithms were used to analyse the vehicle's performance envelope and optimise speed, acceleration and braking for better lap times.

### Software tools:

Python was used for data processing and numerical analysis, with libraries such as NumPy, Pandas and SciPy supporting data processing and optimization. Matplotlib and Seaborn were used for visualisation of results to facilitate data interpretation and decision making.

## RESULTS

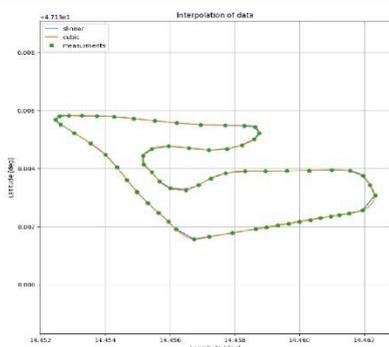


Figure 5: Interpolation of GPS coordinates.

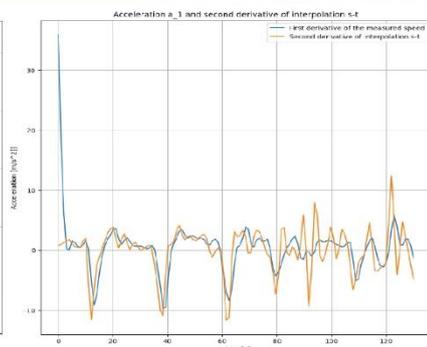


Figure 6: Derivation of measured speed and measured travelled data.

Figure 6 illustrates the comparison between the acceleration obtained by differentiating the measured speed and the acceleration calculated by differentiating twice the distance travelled. It is important to note that the time stamps for both data sets must match to allow an accurate comparison. The measured speed was determined using the phone's GPS data and the Race Chrono app, while the acceleration derived from the distance travelled was calculated by double differentiating using the time and distance data from the car's movement. When comparing the two accelerations, we found that they were broadly similar, although there were some noticeable anomalies. The anomaly at 100 seconds could be attributed to the nature of the differentiation process, which can amplify errors. In addition, the GPS signal may have been weak or disturbed by nearby objects, which could have contributed to these deviations. The significant drop at the beginning of the first derivative of the measured speed is due to the fact that we were at the end of a long straight line before we started the measurement.

In this part of the task (Figure 5), the shape of the racetrack is interpolated using a parametric approach. Unlike conventional 2D curves, which are represented by polynomials, parametric curves allow multiple values of y to correspond to a single value of x. Therefore, to accurately model the shape of the racetrack, a parametric function defined by the new relation  $(x, y) = f(d)$  is introduced, where d is the cumulative distance between consecutive points along the curve. This allows the interpolation to be performed relative to the distance d, providing greater flexibility in accounting for the nonlinear characteristics of the shape of the track.

## CONCLUSION

This study successfully demonstrates the application of numerical methods in analysing the dynamics of racing cars using GPS data. By importing data from the Race Chrono app, which contains detailed information about speed, time and position, we were able to accurately reconstruct the tracks and analyse the movement of the car on the track. This allowed us to identify the key acceleration and braking points that are crucial for optimising the car's performance. Through the driver's feedback, we realised that our ideal line was quite optimal and therefore did not need to be improved. We uncovered specific performance limitations, particularly in cornering and braking, which highlighted areas where the car's dynamics could be refined for better lap times. The methodology developed in this research not only provides actionable insights to optimise vehicle and driver performance, but also provides a solid framework for future studies in the field of motorsport engineering. This work contributes to the growing field of motorsport analytics and provides practical tools to improve competitive performance and achieve faster lap times.



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# On the Way to Renewable Energy Sources: Wind Energy and Wind Farms



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Wind energy is one of the fastest growing forms of renewable energy and a key factor in the transition to a sustainable future. Its environmental and economic benefits are attracting increasing scientific and social attention. Wind as an energy source is inexhaustible, clean and increasingly competitive with fossil fuels, while reducing greenhouse gas emissions and improving energy independence. On this poster we show what a wind turbine consists of, what the opinion polls in Slovenia have shown, why Slovenia must follow the EU's example in wind energy and what goals it has set itself (Kege<sup>et al.</sup>, 2013).

**What makes up a wind turbine?** A wind turbine (**Figure 1**) is a device that converts the kinetic energy of the wind into electricity. A single wind turbine is made of various materials, most of which, or nowadays almost all, can be reused after the lifetime of the individual wind turbine. The materials needed to build a wind turbine are 72% steel, 13% iron or cast iron, 11% fiberglass, resin or plastic, 2% aluminium and 1% copper (Wind Europe, 2024).



Figure 1: Wind turbine.

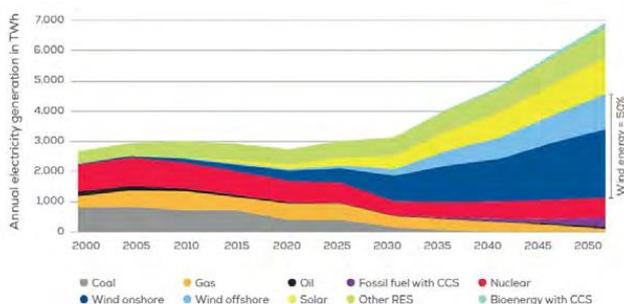


Figure 2: EU Wind Energy Targets (Wind Europe, 2024).

The EU assumes that 50 of energy will come from wind energy and 30 from solar energy by 2050 (**Figure 2**). This is a serious challenge for Slovenia, where we are not even at 1% for wind energy. We are also struggling to meet our renewable energy targets, which are getting higher every year. We are making much better use of solar energy and, to a certain extent, hydropower, but we still have potential in this area too (Wind Europe, 2024).

**How much public support is there for wind energy projects?** In October 2023, Greenpeace Slovenia conducted an opinion poll and the results showed that Slovenians are very positive about wind energy (Greenpeace Slovenia, 2023). 79.6% of respondents stated that they agree or even strongly agree with wind energy. This percentage was even higher among the inhabitants of the Notranjsko-Kraška region, where 86.2% of residents were in favour (Greenpeace Slovenia, 2023).

On October 17, 2024, a presentation of the initiative for a National spatial plan for the Ilirska Bistrica wind farm was held, which was attended by around 300 people (**Figure 3**). Although the media gave the general impression that all those present were against the wind farm, the questionnaire was distributed at the event. The questionnaire included the question of whether people support the possibility of spatialization of the PVE Ilirska Bistrica. 39 people answered the question, 14 of whom were in favour of the proposal and 25 against. It turned out that 36% of participants supported the project in the initial phase.



Figure 3: Presentation at the public event (Photo: M. A. Kege<sup>l</sup>).

## Conclusion

People often say that they are not against wind energy, but they do not want it in their "backyard". They oppose it for environmental, aesthetic, social and economic reasons ranging from visual blight and landscape destruction to concerns about noise, impact on the environment, wildlife and human impacts, negative impact on their property values and often worry about future decommissioning or recycling. These fears are usually based on a lack of knowledge about the area, on the other hand many studies have already disproved most of these fears. Practise shows that this is the case, as there are over 100,000 wind turbines in Europe alone, with which local communities generally coexist and benefit, some of them very well. Further activities, including educating the general public, will follow.

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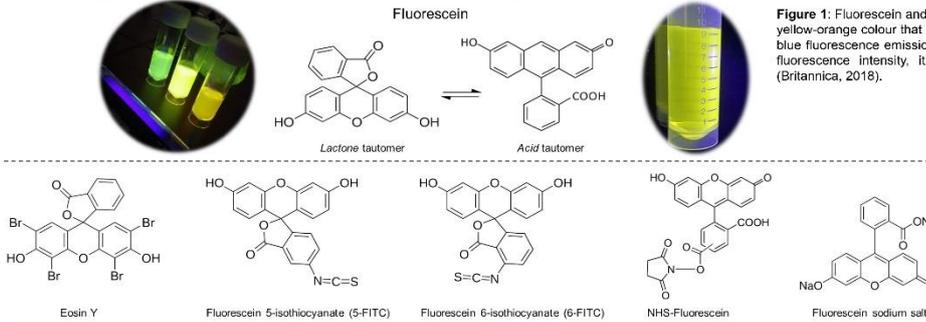


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Fluorescent dyes, which offer a unique way to visualize and analyze complex biological systems, have become an indispensable tool in various scientific and medical fields. Fluorescent molecules, including fluorescein, absorb light at a shorter wavelength and emit light at a longer wavelength. Among these colours, those with a high intensity of fluorescent light emission have a wide applicability, and so we evaluate fluorescein and its derivatives (Figure 1) (Jun et al., 2020; Marinko et al., 2024).



**Figure 1:** fluorescein and its analogues. Fluorescein is a solid organic fluorophore of yellow-orange colour that absorbs at 460 nm when exposed to light and emits green-blue fluorescence emission at 515 nm (Marinko et al., 2024). Due to its exceptional fluorescence intensity, it can be detected even at a dilution of 1:50,000,000 (Briannica, 2018).

**Brain tumors**

Before a craniotomy, in which part of the bone is removed to gain access to the brain, patients are given an injection of fluorescein sodium salt. During the procedure, the cerebral cortex is examined under UV-light; superficial tumors turn yellow-green, which facilitates their identification and delineation. For deeper tumors, biopsy material is used and examined for the appearance of fluorescence, which indicates the presence or absence of diseased tissue (Moore et al., 1950).

**Mouth cancer**

The fluorescein sodium salt was applied topically to the diseased lesion and its surroundings; the mouth was rinsed and the lesion was examined for fluorescence. Fluorescein has been shown to allow rapid and non-invasive identification of potentially malignant disorders (OPMD) in oral squamous cell carcinoma (OSCC), as it can differentiate between healthy oral mucosa and oral dysplasia/malignancy (Kaiser et al., 2020).

**Stomach cancer**

About 10 seconds after injection of a fluorescent marker (fluorescein sodium salt), a rapid spread of fluorescence over the inner surface of the stomach was observed. Early-stage gastric cancer with a higher number of stromal cells showed more pronounced fluorescence than cancer with fewer stromal cells, which showed weaker fluorescence. Stronger fluorescence was also seen in undifferentiated cancers with extensive stroma due to malignant invasion. The borders of early-stage cancer were clearly visible, and some concomitant pathologic changes that had been missed at routine endoscopy were detected at surgery (Bhunchet et al., 2002).

**Breast cancer metastasis to the brain**

It has been shown that surgery with fluorescein sodium salt (5 mg/kg, intravenously; after an allergy test and general anaesthesia) improves the visibility of the tumor and has a better effect on the patient's condition than standard microsurgical treatment. In addition, overall survival after treatment of brain metastases was slightly higher in patients who received a fluorescent marker, indicating the safety and feasibility of the procedure to remove breast cancer brain metastases (Xiao et al., 2018).

**Bladder cancer**

The concept of photodynamic diagnostics (PDD) uses fluorochromes, which emit fluorescence when light is absorbed and thus enable perfect placement of the tumor tissue. In cancer cells, 5-aminolevulinic acid (ALA) promotes the accumulation of fluorescent protoporphyrin IX (PPIX), which creates a contrast between red fluorescent malignant lesions and non-fluorescent normal mucosa (Zaak et al., 2005).



**Figure 2:** Sodium disulfonate salt as a water-soluble fluorescence-active fluorescein analogue (SUF) and its activity in labelling urothelial urinary bladder cancer cells (Jeran et al., 2019).

We have shown experimentally that the compound does not damage the cells and can differentiate between normal and cancerous urothelial cells.

Due to its chemical structure, it was found to be non-toxic and can be used as a cell marker.

Examination of the cells with a fluorescence microscope showed cells at different stages of cell division, confirming that SUF does not inhibit cell division (Figure 2).  
The compound SUF is not cytotoxic.

**LYMPHOBLASTIC AND MYELOID LEUKAEMIA**

Fluorescent dyes are valuable tools in leukaemia research, particularly for identifying and characterizing multidrug resistance (MDR) and targeting specific leukaemia cell populations. These dyes can help in both diagnostic imaging and therapeutic applications (Figure 3).

**Multidrug resistance (MDR) and fluorescent dyes**

**Characterization of MDR:** Fluorescent dyes are used to study MDR in leukaemia cells, such as the P388 murine leukemia and its resistant subline P388/ADR. Differences in dye accumulation can indicate MDR, as seen with cationic dyes like rhodamine esters and cyanines, which are substrates for the drug transport system in resistant cells (Kessel et al., 1991). Similarly, human leukaemia cell lines like K562 and CCRF-CEM have been studied using dyes such as Hoechst 33342 and rhodamine 123 to identify MDR phenotypes (Lautier et al., 1993).

**Efflux mechanisms:** The MDR1 protein is a key player in drug resistance, actively extruding drugs and dyes from leukaemia cells. This efflux can be detected using flow cytometry, which correlates dye efflux with MDR1 expression. However, some cases show efflux without MDR1 expression, suggesting alternative mechanisms (Leith et al., 1995). The dye BCECF is used to study efflux in MRP-overexpressing cells, indicating that MRP mediates substrate removal from the cytoplasm (Draper et al., 1997).

**Targeted imaging and therapy**

**AML targeting:** A near-infrared dye, IR-26, targets acute myeloid leukaemia (AML) cells by accumulating in mitochondria and impairing oxidative phosphorylation. This dye allows for real-time monitoring and targeted therapy, potentially improving treatment outcomes by preventing disease relapse (Zhang et al., 2021).

**Leukemia stem cells:** The fluorescent dye DiOC5(3) selectively marks leukaemia stem cells and inhibits their proliferation. It accumulates in mitochondria and induces apoptosis, offering a dual role in diagnostic imaging and therapy (Zhang et al., 2015).

**Therapeutic applications**

**Photosensitization:** Merocyanine 540 is a fluorescent dye used in photosensitization to selectively kill leukemia cells while sparing normal hematopoietic stem cells. This approach is promising for purging tumor cells from bone marrow grafts (Sieber et al., 1984; Mumcuoglu et al., 1989).

**Challenges and considerations**

**Microenvironmental Contamination:** Lipophilic dyes used for cell tracking can transfer to neighboring cells, leading to contamination. This highlights the need for careful validation when using non-genetically encoded markers for cell tracking (Lassally et al., 2010).

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**Figure 3:** Fluorescent dyes are crucial in leukaemia research for identifying MDR, targeting specific cell populations, and developing therapeutic strategies. However, challenges such as dye efflux mechanisms and microenvironmental contamination must be addressed to optimize their use in clinical applications.

**Conclusion**  
The inclusion of fluorescent dyes in the diagnosis and treatment of cancer represents a significant translation from the scientific to the clinical pathway. Such dyes improve the visibility of tumors, enable early detection by non-invasive methods and improve the accuracy of surgical resections. In practice, these types of compounds need to be used in conjunction with other diagnostic tools or molecular markers to ensure accurate detection of cancer cells and avoid false-positive results from normal but damaged or inflamed tissue.

# GROWTH OF THE GREEN MICROALGAE *CHLORELLA VULGARIS* ON GLASS AND FLUORINATED POLYMER CARRIERS



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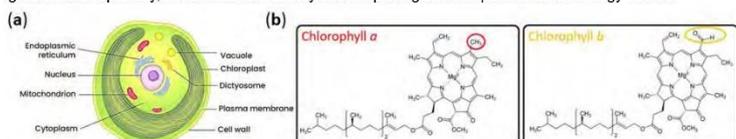
Correspondence: M.J. [marko.jeran@ijs.si](mailto:marko.jeran@ijs.si)



## OUTLINE

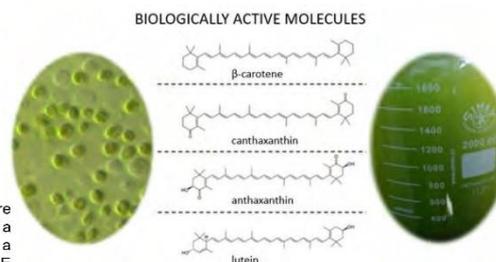
Organisms that are capable of photosynthesis are generally classified as algae, but cannot be classified as higher plants. Algae thrive in many aquatic environments, on the bottom and on rocky substrates. They can swim freely, adhere to various surfaces or grow floating on the water surface. They can even occur in porous rocks, on the surface crust of desert soils, in extremely dry or salty environments (Jeran *et al.*, 2020).

*Chlorella vulgaris* (*C. vulgaris*), one of the best-studied algae, is a eukaryotic green alga (Figure 1). A single chloroplast fills most of the cell. Under stress conditions, lipid droplets form in the cytoplasm and starch granules form in the chloroplast. An algae cell is surrounded by a solid cell wall that changes with the cell cycle; it reproduces asexually. Due to its simple cell cycle and rapid growth, *Chlorella* has long been used as a model microorganism to study photosynthetic and metabolic processes similar to those of higher plants (Jeran *et al.*, 2020). Algae are basically photoautotrophic organisms, *i.e.* they are able to convert light energy into chemical energy through photosynthesis. Under certain conditions, some species can also grow heterotrophically, which means that they use simple organic compounds as an energy source.



**Figure 1:** (a) Organelle of the green microalgae *C. vulgaris* (Allen, *n. d.*) and (b) the chemical structure of chlorophyll (Jeran *et al.*, 2020).

Algae are a rich source of amino acids that our body cannot produce itself (essential amino acids). They are also a source of fatty acids, minerals, vitamins and contain many polyphenols and fiber. *C. vulgaris* which has a nutritional value of about 3 kcal/g, contains about 55% protein, 15% carbohydrates and 10% lipids. It is a source of 9 essential and 5 non-essential amino acids and contains important vitamins such as B<sub>1</sub>, B<sub>2</sub>, B<sub>12</sub>, K, E and C as well as nicotinic acid (De Andrade & Andrade, 2017). Algae have an anti-inflammatory effect, improve immunity and inhibit the occurrence of age-related diseases such as cardiovascular disease, high blood pressure and cataracts. Their extracts are often added to skin care products as they have a moisturizing and rejuvenating effect. Algae extracts are also found in cough remedies and wound healing products (Thiyagarasaiyar *et al.*, 2020). *C. vulgaris* contains biologically active the following carotenoids (yellow-orange pigments):  $\beta$ -carotene, lutein, astaxanthin, canthaxanthin and zeaxanthin (Figure 2).



**Figure 2:** Biologically active molecules in algal biomass (Jeran *et al.*, 2020).

The research work deals with the investigation of the influence of the carrier of the growth vessels (glass, fluorinated polymer material) on the production of the model green alga *C. vulgaris*.

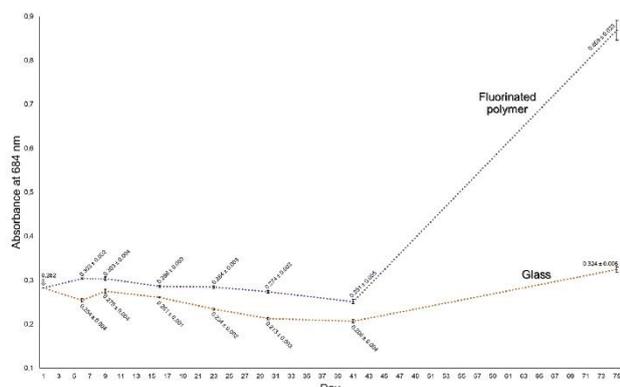
## EXPERIMENTAL METHODS

705 mg of a commercially available medium for the growth of freshwater algae, Bold Basal Medium (BBM) (PhytoTech, USA), was quantitatively transferred into 1 L volumetric flask and diluted with mQ-water to the mark. The pH of the well-mixed contents was 6.6. 43 mL of the *C. vulgaris* inoculum was diluted in a 2 L Erlenmeyer flask with 800 mL of fresh medium. The initial absorbance ( $A_0$ ) of the homogeneous suspension of the working culture prepared in this way was 0.282. The 45-mL aliquots of suspension were then transferred into glass and fluorinated polymer culture vessels (Optinova, Sweden). The culture vessels were covered with a piece of absorbent cotton to ensure air exchange and possible exposure to the outside. The cultivation of the model microalgae was carried out in natural light and at a temperature interval between 20 and 24 °C.

The absorbance of the individual samples was determined spectrophotometrically (Hach Lange, DR 3900, Germany) at a wavelength of 684 nm. For the blank, the BBM medium was used as the set-down point of the device. The sample was homogenized before each individual measurement. The absorbance of each sample was measured in three parallel determinations, and the result was expressed as an average value. In addition to the initial culture, absorbance measurements were also taken on the 6th, 9th, 16th, 23rd, 30th, 41st and 75th day. In this way the growth curve results were recorded (Figure 3). Light microscopy was performed using an Eclipse TE2000-S inverted light microscope (Nikon EM CCD, Tokyo, Japan), in conjunction with a Spot boost digital camera system (Visitron Systems). 100  $\mu$ L of the suspension was pipetted into experimental chambers intended for quantitative field analysis and image acquisition under the microscope. The contents were placed in the chambers under the microscope. When the contents to completely settled to the bottom ( $t = 2$  min), the examination was carried out at 10 $\times$  and 25 $\times$  magnification and at 100 $\times$  magnification using immersion oil. The sample fields were recorded using a system of digital cameras connected via a computer system.

## RESULTS

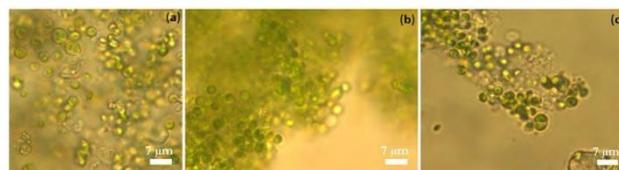
In the first part, we monitored the growing of *C. vulgaris* on two different carriers using absorbance as a function of time (Figure 3). Both tested samples grew uniformly with minimal deviations until day 23. In the transition to the 30th day, a slight decrease in absorption can be seen. Over a further period of 43 days, a strong growth of the cell culture can be seen, which is stronger on the fluorinated polymer carrier. It should be noted that the wavelengths of light correlate with the speed and success of cell proliferation in the culture. The culture in the fluorinated polymer also visually showed a higher yield, as more sediment was visible at the bottom of the container. Despite the fact that we always homogenized the samples before each measurement, the highest standard deviation of the measurements also occurred in the said sample on day 71. The reason for this could be, on a visual level, the appearance of larger cell aggregates and the appearance of agglomerates that tend to settle.



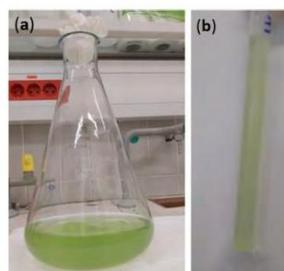
**Figure 3:** Temporal monitoring of the growth of the green microalgae *C. vulgaris* on glass and fluorinated polymer material.

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**Figure 4:** (a) Light microscopy of the initial culture of *C. vulgaris* and its visualization after 75 days on (b) glass and (c) on fluorinated polymer material.



**Figure 5:** Cultures of the microalgae *C. vulgaris* on the 16th day of observation: (a) in an Erlenmeyer flask made of glass and (b) homogenate in fluorinated polymer material.

## CONCLUSION

We have shown that the presence of a fluorinated polymer carrier contributes significantly to the growth and reproduction of the cell culture of the microalgae *C. vulgaris*. From the first to the 30th day after planting, slight fluctuations in absorption can be observed, indicating a slow growth rate and even a decline in some places. Later, on the 75th day of observation, the cells enter a phase of intensive growth and production of biologically active substances. Cell clusters and agglomerates are formed. The cellular biomass in a container of fluorinated polymer takes on non-uniform mathematical forms that differ in concentration per unit volume.

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# Trends in Labor Inductions in Slovenia: Insights from 20 Years of Clinical Data (2002–2022)

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## OUTLINE

Labor induction, a critical aspect of obstetric practice, has long been debated for its timing and implications. The ARRIVE trial (A Randomized Trial of Induction Versus Expectant Management), published in 2018, significantly influenced this discourse, comparing elective induction at 39 weeks to expectant management in low-risk nulliparous women. Key findings highlighted reduced cesarean rates and improved perinatal outcomes in the induction group, challenging traditional practices (James-Contereilli & Kennedy, 2023). However, the trial's applicability outside the U.S. remains uncertain due to demographic and healthcare system differences, particularly in Europe, where maternal profiles and neonatal outcomes differ markedly. Subsequent studies and critiques have further nuanced the conversation, suggesting that while induction has measurable benefits, its implementation requires careful consideration of local contexts and patient preferences (Carmichael and Snowden, 2019; Facchinetti et al., 2022).

In this study, we conducted a comprehensive analysis of the trends and frequency of labor inductions in Slovenia over a two-decade period, spanning from 2002 to 2022.



## STUDY DESIGN & METHODS

The study employed a retrospective cohort design, utilizing comprehensive data from the National Informational Perinatal System (NIPS). This database encompassed detailed records of all labor cases across 14 hospitals in Slovenia over a two-decade span (2002–2022). The design aimed to explore trends in labor induction rates, evaluate their correlation with obstetric practices, and investigate subgroup patterns using the widely recognized Robson classification system. The cohort included all women admitted for labor, ensuring a representative sample of clinical presentations and outcomes across the studied period.

The study aimed to classify labor cases according to a modified Robson classification system based on a set of specific criteria (Rossen et al., 2017). The Robson classification system is a widely accepted method for categorizing women into groups based on characteristics that affect the likelihood of cesarean section. The criteria and classification methods applied in this study are as follows:

- Group 1: Women were assigned to Robson group 1 if they were carrying a singleton fetus in cephalic presentation, at term, had spontaneous labor, and were primiparous.
- Group 2: Women were assigned to Robson group 2 if they were carrying a singleton fetus in cephalic presentation, at term, had labor induced, and were primiparous. This group also includes elective cesarean sections; if these are excluded, it is referred to as group 2A.
- Group 3: Women were assigned to Robson group 3 if they were carrying a singleton fetus in cephalic presentation, at term, had spontaneous labor, and were multiparous without a previous cesarean section.
- Group 4: Women were assigned to Robson group 4 if they were carrying a singleton fetus in cephalic presentation, at term, had labor induced, and were multiparous without a previous cesarean section. This group also includes elective cesarean sections; if these are excluded, it is referred to as group 4A.
- Group 5: Women were assigned to Robson group 5 if they were carrying a singleton fetus in cephalic presentation, at term, were multiparous with a previous cesarean section, regardless of whether labor was spontaneous or induced.
- Group 6: Women were assigned to Robson group 6 if they were carrying a singleton fetus in breech presentation and were primiparous, regardless of gestational age or whether labor was spontaneous or induced.
- Group 7: Women were assigned to Robson group 7 if they were carrying a singleton fetus in breech presentation and were multiparous, regardless of gestational age or whether labor was spontaneous or induced.
- Group 8: Women were assigned to Robson group 8 if they were carrying multiple fetuses.
- Group 9: Women were assigned to Robson group 9 if they had a fetus in transverse lie.
- Group 10: Women were assigned to Robson group 10 if they did not fit into the previous categories and had a preterm birth.

Each woman's data was evaluated according to these criteria, and they were assigned to the appropriate Robson group accordingly. This classification allowed for the standardized comparison of labor induction outcomes across different subgroups.

## RESULTS

An examination of labor onsets in Slovenia over the period from 2002 to 2022 yielded notable findings. Analysis of the data reveals an upward trend in the percentage of labor inductions, including elective cesarean sections, accompanied by a corresponding decrease in spontaneous labor onset. As illustrated in **Figure 1**, the increase in labor inductions commenced prior to the publication of the ARRIVE trial in 2018, suggesting that this trial alone cannot be attributed as a significant factor influencing the observed trend. Nevertheless, 2018 marks the first year in which the overall percentage of inductions (including elective cesarean sections) exceeded 30%. This growth trajectory has shown no signs of abating through 2022.

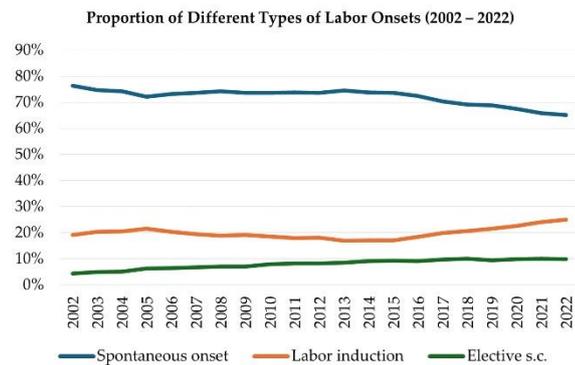


Figure 1: Proportion of different types of labor onsets in Slovenia during the years 2002–2022.

An examination of the distribution of labors in Slovenia across different Robson groups over a 20-year period, as observed in **Figure 2**, reveals a relatively stable overall pattern. Nevertheless, a decline in the proportions of Robson Groups 1 and 3 can be observed, accompanied by a corresponding increase in the proportions of Robson Groups 2 and 4.

The increase of labors, assigned to Robson Group 5 can likely be attributed to the rising number of cesarean sections, resulting in a higher number of women falling into this category in subsequent labors.

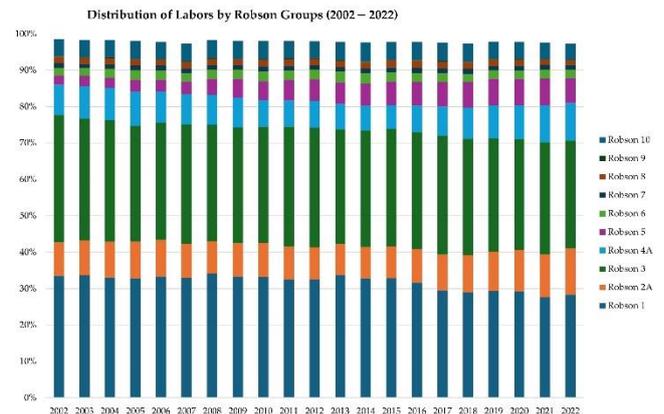


Figure 2: Distribution of labors in Slovenia by Robson groups between 2002 and 2022.

Although Slovenia is not an exception in the medicalization of childbirth, it is impossible to attribute the rise in labor inductions solely to this phenomenon. These trends must be understood within the broader demographic and epidemiological context, wherein women are increasingly postponing their first pregnancies to later stages of life. This delay is accompanied by a heightened incidence of comorbidities and risk factors, shaped by both advanced maternal age and evolving lifestyle patterns, which differ markedly from those observed two decades ago.

## CONCLUSION

The analysis indicates a steady increase in labor inductions in Slovenia, which may be attributed to an aging maternal population and a higher prevalence of chronic illnesses. Reducing the frequency of inductions will rely heavily on comprehensive health education targeting the general population, empowering women from an early age with knowledge about the impact of factors such as maternal age and obesity on childbirth outcomes. With targeted public health initiatives and a proactive approach to promoting healthier lifestyles, there is a promising opportunity to reverse the upward trajectory of labor inductions and improve maternal and neonatal outcomes in the future.

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# RARE EARTH ELEMENTS IN SUSTAINABLE DEVELOPMENT AND BIOMATERIALS FOR BIOLOGICAL APPLICATIONS AND AS A CHALLENGE FOR EDUCATION



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## OUTLINE

The group of metals known as rare earth elements (REE) comprises the elements of the lanthanide series. The metals of the lanthanide series are: Lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb) and lutetium (Lu). In addition, yttrium (Y) and scandium (Sc) are often grouped together with the lanthanides and referred to as REE (Australian Government, 2023).

REE resources are mainly associated with four geological environments: alkaline igneous rocks, carbonates, placer deposits with monazite xenotime mineralization and clay deposits with ion adsorption (Australian Government, 2023).

**Figure 1:** The position of the rare earth elements in the periodic table, i.e. the 17 metallic elements according to the International Union of Pure and Applied Chemistry (IUPAC) (Coit and Dahlgren, 2019).

REE are extremely important components of high technology, which is why they are sometimes referred to as the “vitamins” of the modern economy or industry (Figure 1). With the development of high technology, the demand for rare earth metals is increasing year on year. This is particularly evident in the search for clean energy sources or in the development of various types of electronic devices. In 2021, global production of rare earth elements reached 280,000 tons, a significant increase compared to 2018, when 110,000 tons less were mined (Duchna and Ciešlik, 2022).

## KEY APPLICATIONS OF REE

Adopted from Ramprasad *et al.*, 2022.

### Manufacturing Industries

Metal alloys, ceramic pigments, coloured glassware, polishing powders, additives stress gauges

### Electrical & Electronic Industry

Television, computers, mobile phones, rechargeable batteries, compact fluorescent lamps (CFLs), printers and scanners

### Green and Renewable Sector

Wind turbines, biofuel cells and green automobiles

### Optical Communication Systems

Laser, optical glass, fiber, optic cables, vapor lamps, nuclear batteries and superconductors

### Medical & Scientific Sector

X-ray machines, dental lasers, magnetic resonance imaging (MRI), scanners and cancer equipment

### Others

Hybrid automobiles, powerful magnets and reduce the motor weights

### Luminescent materials with the REE ions most frequently used in biological experiments (Duchna and Ciešlik, 2022).

Matrices	REE ions	Ligands	Applications
NaYF <sub>4</sub>	Yb-Tm, Yb-Ho, Yb-Ho-Ce	—	<i>in vivo</i> imaging
NaYF <sub>4</sub>	Yb <sup>3+</sup> , Er <sup>3+</sup>	NaYF <sub>4</sub> /SiO <sub>2</sub>	Photodynamic therapy
Y <sub>2</sub> O <sub>3</sub>	Er <sup>3+</sup>	PEG	Cancer cells detection
NaYF <sub>4</sub>	Nd <sup>3+</sup>	—	Hiperthermic
LaF <sub>3</sub>	Ce <sup>3+</sup> , Tb <sup>3+</sup>	PSS, PAH	Glucose marking
NaYF <sub>4</sub>	Yb <sup>3+</sup> , Er <sup>3+</sup> , Gd <sup>3+</sup>	<sup>18</sup> F, citric/oleic acid	Magnetic resonance, Emission tomography
Gd <sub>2</sub> O <sub>3</sub> , Fe <sub>3</sub> O <sub>4</sub>	Eu <sup>3+</sup>	DNA	Detection of DNA mutations
LaF <sub>3</sub>	Yb <sup>3+</sup> , Er <sup>3+</sup>	SiO <sub>2</sub>	Transportation of drugs

REE-based biomaterials have representative optical/magnetic properties that show great potential for biomedical applications. In recent years, many studies have shown that rare earth-based biomaterials also possess special biological properties, including antioxidant, anti-inflammatory and antibacterial activity, which also enables their potential application in improving wound healing and skin tissue regeneration. The development of the next generation of smart bioactive dressings and devices based on rare earths should be very interesting and valuable for wound healing (Luo *et al.*, 2022).

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### Recovery of various rare earths from waste electrical and electronic equipment using different methods (adopted from Ramprasad *et al.*, 2022).

E-waste	Method	REE extracted	% Recovery at lab scale	Reference
Waste fluorescent powder	Hydrometallurgical method. Alkaline roasting-acid leaching process for the conversion of Al <sub>2</sub> O <sub>3</sub> into water soluble NaAlO <sub>2</sub> so that it can be easily separated from REE oxides by filtration.	CeO <sub>2</sub> , Eu <sub>2</sub> O <sub>3</sub> , Er <sub>2</sub> O <sub>3</sub> , Tb <sub>2</sub> O <sub>3</sub> , Y <sub>2</sub> O <sub>3</sub>	99.8%	Liao <i>et al.</i> , 2017
Discarded NdFeB magnets	Hydrometallurgical method. Carbonization/hydrogenation -hydrolysis using biochar as an extractant.	Nd, Gd, Pr	88.4%	Liu <i>et al.</i> , 2019
NdFeB battery waste	Hydrometallurgical method. H <sub>3</sub> PO <sub>4</sub> leaching.	Nd, Pr, Dy	98.76%	He <i>et al.</i> , 2021
NdFeB waste	Hydrometallurgical method. H <sub>2</sub> C <sub>2</sub> O <sub>4</sub> /HCl leaching.	Nd, Pr	≤93.89%	Liu <i>et al.</i> , 2021
Waste permanent magnets	Membrane technology. Solvent extraction followed by filtration through a hollow fiber supported liquid membrane.	Nd, Dy, Pr	63.13% (Nd), 15.21% (Dy), 56.29% (Pr)	Ni'am <i>et al.</i> , 2020
Waste permanent magnets	Thermal pre-treatment integrated with the hydrometallurgical process. High temperature roasting, followed by HCl/H <sub>2</sub> SO <sub>4</sub> leaching.	Nd, Dy, Pr	78% (Nd), 83% (Pr), 76% (Dy)	Ni'am <i>et al.</i> , 2019
Computer monitor scraps	Hydrometallurgical method. HCl/H <sub>2</sub> SO <sub>4</sub> leaching.	Eu, Y	greater than 97%	Resende and Moraes, 2010
Permanent magnet waste	Electrochemical method. Electrolysis was undertaken in molten LiF-CaF <sub>2</sub> .	Nd, Pr	Nd (97.25%), Pr (99.1%)	Yang <i>et al.</i> , 2020
Fluorescent powder	Biometallurgical method. Y and Er were leached using <i>Lactobacillus casei</i> and <i>Komagataeibacter xylinus</i> .	Y, Er	5.0% ( <i>L. casei</i> ), 12.6% ( <i>K. xylinus</i> )	Hopfe <i>et al.</i> , 2018
Spent fluid catalytic cracking catalysts	Electrochemical method. Spent fluid catalytic cracking catalysts were leached using HNO <sub>3</sub> at 80 °C followed by removal of Fe from the leach solution via solvent extraction. La and Ce were subsequently extracted from the HNO <sub>3</sub> leach solution by an organic solvent. H <sub>2</sub> SO <sub>4</sub> was used to strip the REEs from the organic phase. Ce and La were separated by a <i>in situ</i> electrochemical oxidation method integrated with simultaneous solvent extraction.	La, Ce	—	Zhou <i>et al.</i> , 2020

## CONCLUSION

With the digitalization of the developed world and new medical technologies, REEs are now considered strategic metals in the global economy. Their use is therefore of interest in various fields, from engineering to medicine. In medicine, they have become important for various molecular imaging and radiotherapy procedures, and in interdisciplinary science, they are a topic of recycling and reuse in the transition to a circular economy.

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