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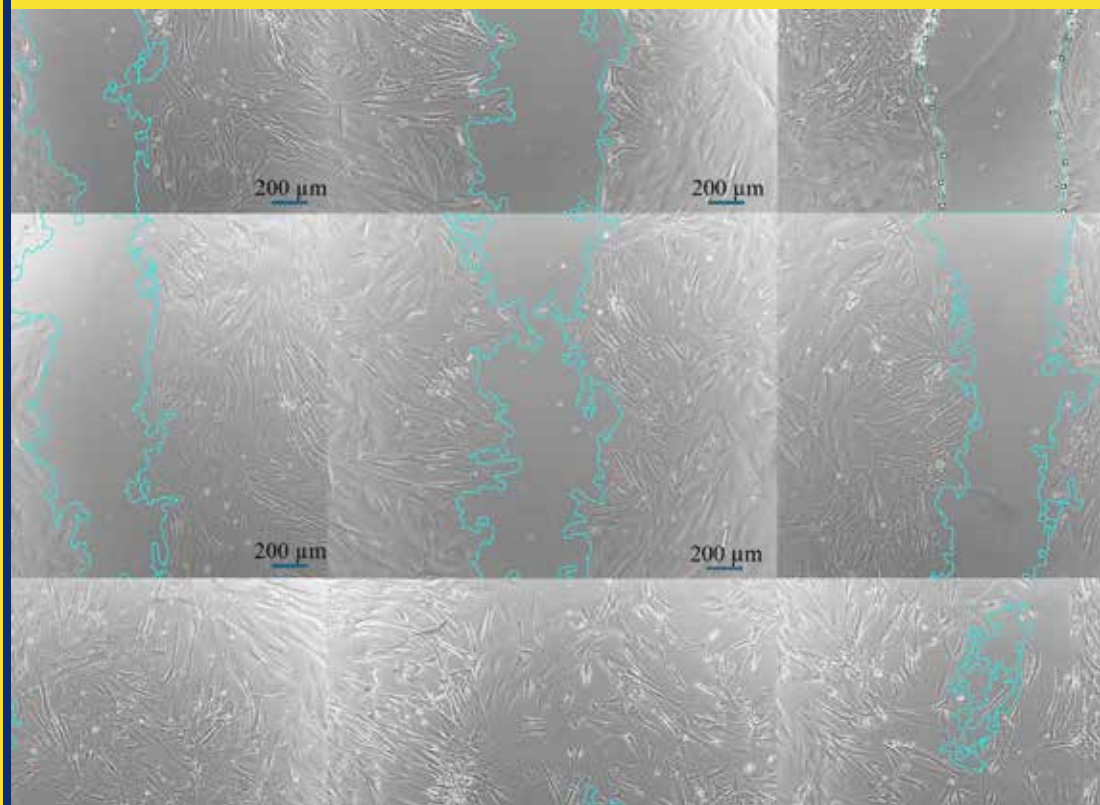
acta MEDICO-BIOTECHNICA



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Reference:
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Acta medico–biotechnica je uradna znanstvenoraziskovalna strokovna revija Medicinske fakultete Univerze v Mariboru, ki je namenjena širšemu krogu bralcev, saj pokriva področje medicine in biotehnike.

Revija bo izhajala najmanj dvakrat letno, v njej objavljene prispevke pa bo strokovno recenziral mednarodni uredniški odbor.

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Uredniški odbor ima pravico odločiti, ali je prispevek sprejet v objavo, določiti recenzente preko mednarodnega uredniškega odbora in, če je potrebno, zahtevati spremembe v vsebini, dolžini ali jeziku.

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Vsi zaključki, mnenja in podatki, objavljeni v prispevku, so izključno delo avtorja in ne izdajatelja ali uredništva. Izdajatelj in uredniki ne sprejmejo nobene kazenske odgovornosti za kakršne koli napake, ki bi bile tako storjene.

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Za čim učinkovitejšo in hitro vodenje postopka se vsi prispevki posredujejo uredništvu revije po elektronski pošti na naslov **actamedbio@um.si**.

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Uvodnik: do 1000 besed, izvleček ni potreben, največ pet referenc, le na povabilo urednika.

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Klinična študija: do 3500 besed, strukturiran izvleček z največ 250

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Poročilo o primeru: 750 besed, strukturiran izvleček (Namen, Poročilo o primeru, Zaključek) z največ 200 besedami, maksimalno 12 referenc.

PRIPRAVA PRISPEVKA

Prispevek mora biti napisan v angleškem jeziku, z izvlečkom v slovenskem in angleškem jeziku, na papirju velikosti A4 (210 x 297 mm) s 3 centimetrskimi stranskimi robovi in dvojnim presledkom med vrsticami. Strani prispevka morajo biti oštevilčene.

Priporočena dolžina je 15 tipkanih strani (6 tiskanih strani). Članek mora biti shranjen v svoji originalni obliki (Microsoft Word for Windows), ves slikovni material pa v posebni datoteki. Tekst mora biti jasno razdeljen na naslednja poglavja:

Izvleček, Uvod, Material in metode, Rezultati in Razprava.

Naslovna stran

Vsebovati mora:

- natančen in jasen naslov,
- ime in priimek avtorjev,
- ime in naslov ustanov avtorjev,
- naslov za dopisovanje pristojnega avtorja (e–mail naslov, telefon in številka faksa).

Druga stran

- **Izvleček:** strukturiran izvleček mora vsebovati osnovne značilnosti prispevka v logičnem in jasnem zaporedju. Podati mora naslednje informacije: namen, metode, rezultate in zaključek. Izvleček ne sme presegati 250 besed.
- **Ključne besede:** največ 3–6 besed, pomembnih za indeksiranje.

Tekst

Tekst naj bo razdeljen na naslednja poglavja: Uvod, Material in metode (vključno z izjavo Etične komisije, kjer je to potrebno), Rezultati, Razprava z zaključki, Zahvala in Literatura.

- **Uvod:** v uvodu je potrebno navesti namen raziskave in posredovati kratek pregled literature zaradi boljšega razumevanja in ocenjevanja dobljenih rezultatov.
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- Če gre v prispevku za poskus na živalih ali prostovoljcih, je potrebno na koncu poglavja Material in metode vključiti izjavo »Potrjujem–o, da je bila raziskava opravljena v skladu z etičnimi načeli in zakonodajo.«
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- **Slikovno gradivo:** ilustracije, diagrami in fotografije morajo biti označeni s številko, in sicer v zaporedju, v katerem se pojavljajo v tekstu. Legende ilustracij in diagramov naj bodo kratke, vendar z zadostnimi podatki, napisanimi na koncu prispevka na posebnem listu. Črno–bele in barvne fotografije morajo imeti resolucijo od 300 dpi, linijske risbe od 800 do 1.200 dpi. Poslane morajo biti v posebni elektronski datoteki. Objava šestih ali manj barvnih fotografij/ilustracij je brezplačna.
- **Tabele:** ob tabelah se morajo navesti legenda, naslov in pojasnilo vseh uporabljenih okrajšav. Vsaka tabela se mora nahajati na posebni strani, biti mora oštevilčena, naslovljena in citirana v tekstu. Tabele so lahko oblikovane v Wordu ali podane v posebni elektronski datoteki.

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Korekcija članka bo poslana v PDF datoteki po e–pošti avtorju, ki je pristojen za dopisovanje. Avtor mora označiti napake, preveriti, ali je besedilo popolno in ali so vključene vse fotografije in tabele. Krtačni odtis je potrebno čim prej vrniti uredništvu. Spremembe v odtisu z izjemo tiskarskih napak niso možne, razen s posebnim dovoljenjem odgovornega urednika.

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Title Page

- A concise and informative title
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Second page

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Please use the following headlines: Introduction, Material and methods (including Statement of Ethics where applicable), Results, Discussion with Conclusions at the end, Acknowledgements (if any), References.

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ACTA MEDICO-BIOTECHNICA

KAZALO / CONTENTS

Nagovor rektorja prof. dr. Zdravka Kačiča ob rektorjevem dnevu Univerze v Mariboru

29. januar 2025

Spoštovani gostje rektorji in prorektorji slovenskih univerz, spoštovana akademska skupnost Univerze v Mariboru: nagrajenke in nagrajenci, prorektorji, dekanice in dekani fakultet, zaslužni profesorji in upokojeni profesorji, učitelji, raziskovalci in strokovni sodelavci, študentke in študenti, gospe in gospodje!

Letošnje leto je za Univerzo v Mariboru posebno, jubilejno leto. Pred 50 leti je bila dne 18. septembra 1975 »ob zvoku fanfar v zboru uglednih gostov in domačinov slovesno razglašena druga slovenska univerza«. Z današnjo slovesnostjo pričenjamo sklop celoletnega praznovanja 50. obletnice ustanovitve Univerze v Mariboru.

V osebnem življenju posameznika srečanje z »abrahamom« običajno predstavlja nekakšen ustvarjalni vrh oz. polno zrelost pred počasnim prehodom v starost. To pa ne velja, vsaj ne nujno, za obletnico institucij, še posebej ne glede prehoda v starost in še posebej ne za institucijo, kot je Univerza v Mariboru, ki je polna vitalnosti in smelih načrtov usmerjena v jutri.

Z jasnim pogledom v prihodnost smo sprejeli strateške usmeritve. Zastavili smo si ambiciozne a realne cilje, z doseganjem katerih bomo pomembno nadgradili kakovost našega izobraževalnega in raziskovalnega dela ter prenosa znanja v okolje. Zastavljeni cilji za trajnostni razvoj univerze sledijo smeri razvoja bolonjske reforme, z namenom, da še bolj utrdimo in zagotovimo konkurenčno znanje na globalni ravni.

Trajnostnost postaja osrednja tema delovanja. Naše izobraževalne in raziskovalne dejavnosti so usmerjene v doseganje trajnostnih ciljev, kot so varovanje okolja, socialna vključenost in ekonomska odpornost. Kot univerza, se zavedamo svoje odgovornosti pri oblikovanju bodočih generacij strokovnjakov, ki

Address of Rector Prof. dr. Zdravko Kačič on Rector's Day at University of Maribor

January 29, 2025

Revered Guest Rectors and Vice-Rectors of Slovenian universities, Revered Academic Community of the University of Maribor: Prize Winners, Vice Rectors, Deans of Faculties, Emeritus Professors and Retired Professors, Teachers, Researchers and Associate Experts, Students, Ladies and Gentlemen!

This year is a special Jubilee year for the University of Maribor. Fifty years ago, on September 18, 1975, "a second Slovenian university was solemnly proclaimed upon the sound of a fanfare in an assembly of distinguished guests and locals." With today's ceremony, we begin a year-long celebration to mark the 50th anniversary of the founding of the University of Maribor.

In an individual's personal life, the meeting with "Abraham" usually represents a sort of creative peak, signaling full maturity prior to a slow transition to old age. This does not apply—at least not necessarily—to the anniversary of institutions, and especially not to the transition to old age or to an institution such as the University of Maribor, which is full of vitality and bold plans, being future-oriented.

With a clear view towards the future, we have adopted strategic directions. We have set ambitious but realistic goals. By achieving these goals, we will upgrade the quality of our educational and research work and our knowledge transfer to the environment. We have defined goals for the sustainable development of the university and followed the development directions of the Bologna Reform with the aim of further consolidating and ensuring competitive knowledge on a global scale.

Sustainability is becoming a central theme of our function. Our educational and research activities are directed towards the achievement of sustainable goals such as environmental protection, social inclusion and

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bodo s svojim znanjem in vrednotami ustvarjali bolj pravično in trajnostno družbo. Nadaljevali bomo z aktivnostmi za uresničitev zastavljenih ciljev do konca desetletja in zagotovili končno izvedbo vseh načrtovanih projektov za ustrezno infrastrukturo vsem članicam Univerze v Mariboru.

Zato je pomembno, da tudi v prihodnjih letih zagotovimo ustrezno kontinuiteto našega skupnega dela, ki bo temeljilo na že doseženem. Ob vzajemnih naporih, da bi ustvarili boljšo prihodnost, bo zlasti treba okrepiti medsebojno zaupanje, znotraj tako posameznih fakultetnih skupnosti kot tudi celotne univerze. Za vzor pri tem imamo lahko pionirje mariborskega visokega šolstva, ki so se nekdaj skupaj kot uporniki oziroma »zaporožci«, kot so se sami poimenovali, pogumno in uspešno borili za t. i. »mariborski koncept višjega šolstva v takratnem reformiranem visokem šolstvu« oziroma za pravico do znanstveno-raziskovalnega dela, kar je omogočilo nadaljnji razvoj visokega šolstva v Mariboru.

Ob tem želim izpostaviti pomen sožitja med posameznikom in skupnostjo, kar predstavlja pomemben vidik poslanstva univerze. Beseda univerza izhaja iz latinskega izraza »universitas magistrorum et scholarium«, kar v grobem prevodu pomeni »skupnost učiteljev in študentov«. Prav tako je tudi vsaka oseba, ki obiskuje univerzo, torej vsak študent in študentka, ki prestopi prag univerze in jo obiskuje nekaj let, sam po sebi univerzum. Vsak posameznik namreč tvori majhen delček vesolja in v vsakem posamezniku biva celoten univerzum.

Na univerzi se torej srečata dva univerzuma; univerzum sveta in znanja ter univerzum človeka – ne človeka na splošno, ki ne obstaja, ampak ravno tiste osebe, mladega človeka, s svojo zgodovino in osebnostjo, sanjami ter intelektualnimi, moralnimi in drugimi lastnostmi. Izziv univerze je ti obzorji, svetovno in osebno, povezati, da se bosta lahko pogovarjali, in bo iz tega pogovora izšla osebnostna rast samega študenta, ki se oblikuje, dozoreva v znanju in svobodi, v sposobnosti razmišljanja in delovanja, kritičnega in ustvarjalnega sodelovanja v družbenem in civilnem življenju, z lastno kulturno in strokovno kompetenco.

economic resilience. As a university, we are aware of our responsibility to shape future generations of experts who shall, by virtue of their knowledge and values, create a more righteous and sustainable society. We shall continue to engage in activities that will help us to realize our set goals and ensure the final implementation of all planned projects by the end of the decade, with a view to providing appropriate infrastructure to all members of the University of Maribor.

This is why it is important that we also promote the continuity of our joint efforts in the years to come, by ensuring that they are rooted in what has already been achieved. In building upon our mutual efforts to create a better future, it will be crucial to strengthen mutual trust within individual faculty communities, as well as across the entire university. As a role model to guide us on our path forward, we can look to the pioneers of Mariborian Higher Education who were once rebels—or "Zaporozhians", as they called themselves—who bravely and successfully fought together for the so-called "Mariborian concept of higher education in the then reformed higher education" or for the right to undertake scientific research which facilitated further development of higher education in Maribor.

With this in mind, I would like to highlight the importance of the coexistence that is found between the individual and the community which is an essential aspect of the university's mission. The word "university" originates from the Latin term universitas magistrorum et scholarium, which roughly means "community of teachers and students." Every person who attends a university is a student who crosses its threshold and attends it for a few years; in this way, they experience their own universe. Each individual forms a small particle of outer space or universe, and in every individual, the entire universe dwells.

At a university, two universes meet: the universe of the world and knowledge, and the universe of a human-being. We do not speak of a human-being in a general sense, but rather the specific person, the young human-being with his/her history and his/her personality, his/her dreams and intellectual, moral and other characteristics. The challenge of the university is to connect these two horizons; that of the world and the personal, so that they can speak to each other. From this dialogue, the student's

Univerza tako na nek način predstavlja privilegiran kraj srečevanja, ob nenehnem iskanju človeške harmonije ter trudu za graditev mostu med sedanjostjo in prihodnostjo, med potencialom in uresničitvijo, med izzivi in rešitvami. Naša odgovornost je, da ta most nenehno dograjujemo in utrjujemo, da bo vsak korak čez varen, vzdržljiv in usmerjen v boljši svet.

Spoštovani, vsako leto znova ob Rektorjevem dnevu izpostavljamo dosežke, uveljavitev in razvoj Univerze v Mariboru, in sicer na način, da podelimo nagrade in priznanja tistim posameznikom, za katere smo v njihovem delu in dosežkih prepoznali pomemben prispevek za celotno akademsko skupnost. Vsem letošnjim nagrajencem in dobitnikom priznanj iskreno čestitam, ob čemer se zavedam, da je še mnogo drugih, ki s svojim delom doprinašajo k ugledu te univerze, zato se jim prav tako zahvaljujem. Podeljena nagrada naj bo vzpodbuda, v zavedanju, da se je za znanje potrebno potruditi. Zaslužena priznanja za vaš dragoceni prispevek naj vas navdihujejo na nadaljnji poti, vse nas pa vzpodbujajo k temu, da še naprej krepimo zavezanost humanizmu, svobodi, solidarnosti in enakosti kot osnovnim vrednotam, ki uokvirjajo in pogojujejo naše delo.

Predvsem pa naj nikoli ne usahne naš notranji zagon za iskanje resnice, odgovorov in znanja, kar pomembno doprinaša k samouresničevanju človeka, njegovi etični drži in s tem boljši družbi kot celoti.

Prof. dr. Zdravko Kačič
rektor Univerze v Mariboru

personality grows, emerges and matures, as they develop knowledge and freedom, the ability to think and behave, and to cooperate critically and creatively in social and civil life, and within the scope of his/her own cultural and professional competence.

So in some way, the university represents a privileged meeting place wherein we strive constantly to achieve human harmony and to build a bridge between the present and future, between potential and realization, between challenges and solutions. It is our responsibility to keep building and fortifying this bridge so that each step over it shall be safe, durable and directed to a better world.

Revered Guests, each year on Rector's Day, we highlight the achievements, establishment and development of the University of Maribor. We award prizes and recognitions to individuals whose works and achievements have contributed significantly to the entire academic community. I offer my sincere congratulations to all of this year's award winners and recipients, and to the many others I am aware of whose efforts nurture the reputation of the university. And for this reason, I also express my gratitude to them. The given award should make us mindful that knowledge is acquired through effort. The deserved recognitions for your valuable contribution should inspire you on your future paths and encourage us all to further strengthen our commitment to humanism, liberty, solidarity and equality as basic values which frame and condition our work.

Above all, may our inner momentum to seek truth, answers and knowledge never wither, because it is these aspirations that guide the self-realization of an individual as a human, his/her ethical stance, and the betterment of society as a whole.

Prof. dr. Zdravko Kačič,
Rector of University of Maribor

Pristop k obravnavi in zdravljenju otrok s kroničo boleznijo ledvic

Management and treatment of children with chronic kidney disease

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Izvleček

Kronična ledvična bolezen (KLB) je definirana kot motnja ledvične funkcije ali strukture, ki je prisotna več kot tri mesece, in ima pomemben vpliv na zdravje s tveganjem za postopno izgubo ledvične funkcije. Pred kratkim so bila objavljena nova priporočila o obravnavi bolnikov s KLB. Osnovna obravnava otrok s KLB vključuje spodbujanje zdravega življenjskega sloga, preprečevanje napredovanja KLB, zdravljenje zapletov KLB ter pripravo bolnika in njegove družine na nadomestno ledvično zdravljenje, ko je potrebno. Vzdrževanje zdravega življenjskega sloga vključuje natančno spremljanje ledvične funkcije in morebitnih zapletov, spremljanje rasti, prehrane, nevrološkega razvoja in krvnega tlaka. V okviru preprečevanja napredovanja KLB

Abstract

Chronic kidney disease (CKD) is defined as a disorder of kidney function or structure that is present for more than 3 months and has a significant impact on health with the risk of progressive loss of kidney function. New recommendations for the management of patients with CKD have recently been published. The basic management of children with CKD includes promoting a healthy lifestyle, preventing the progression of CKD, treating associated complications, and preparing the patient and their family for renal replacement therapy if needed. Maintaining a healthy lifestyle includes close monitoring of kidney function and potential complications, monitoring growth, nutrition, neurological development, and blood pressure. In the context

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je pomembno zdravljenje osnovne bolezni, če je mogoče, ter preprečevanje nadaljnje akutne poškodbe ledvic. Dobro urejen krvni tlak in kontrola proteinurije dokazano upočasnjujeta napredovanje ledvične bolezni pri otrocih in sta zato življenjskega pomena. KLB pomembno vpliva na kakovost življenja otroka in njegove družine. Celostna obravnava otroka vključuje obravnavo v multidisciplinarnem timu, ki deluje proaktivno.

of preventing the progression of CKD, it is important to treat the underlying disease, if possible, and prevent further acute kidney injury. Well-controlled blood pressure as well as proteinuria have been shown to slow the progression of kidney disease in children, and are therefore vital. CKD significantly affects the quality of life of a child and his family. Comprehensive treatment of a child includes treatment by a multidisciplinary team that works proactively.

INTRODUCTION

Chronic kidney disease (CKD) in children is a long-term condition characterized by increased risk of gradual loss of kidney function over time. The Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group has recently updated the definition, which describes an abnormality of kidney structure or function, present for a minimum of 3 months, with implications for health (1). To define CKD, one or more markers of kidney damage should be present: albuminuria (albumin-to-creatinine ratio ≥ 30 mg/g or ≥ 3 mg/mmol), urine sediment abnormalities, persistent haematuria, electrolyte and other abnormalities due to tubular disorders, abnormalities determined by histology, structural abnormalities detected by imaging, or a history of kidney transplantation. Decreased glomerular filtration rate (GFR) < 60 ml/min/1.73 m2 without other markers of kidney damage is also defined as CKD (1). The classification system is based on cause, GFR (G1–G5), and albuminuria category (A1–A3). Both categorical classifications are presented in Table 1, summarized from the recent published guidelines (1). It is important to note that healthy children and adolescents should have excellent kidney function; therefore, even an estimated GFR of only stage G2 indicates decreased kidney function and poses a threat to overall health across the lifespan (1).

While CKD is more commonly associated with adults, it significantly affects the paediatric population. Epidemiological studies of all-stage CDK in children

Table 1: Glomerular filtration rate and albuminuria categories in defining chronic kidney disease according 2024 Kidney Disease: Improving Global Outcomes guidelines (1); GFR – glomerular filtration rate; CKD – chronic kidney disease.

GFR (ml/min/1.73 m²) categories in CKD		
G1	≥ 90	Normal or high
G2	60 – 89	Mildly decreased
G3a	45 – 59	Mildly to moderately decreased
G3b	30 – 44	Moderately to severely decreased
G4	15 – 29	Severely decreased
G5	< 15	Kidney failure
Albuminuria categories in CKD		
A1	< 30 mg/24 h or < 3 mg/mmol or < 30 mg/g	Normal to mildly increased
A2	30 – 300 mg/24 h or 3 – 30 mg/mmol or 30 – 300 mg/g	Moderately increased
A3	> 300 mg/24 h or > 30 mg/mmol or > 300 mg/g	Severely increased

Table 2: Aetiology of chronic kidney disease in children, categorized by congenital and acquired causes.

Aetiology type	Aetiology group of causes	Examples
Congenital	Congenital anomalies of the kidney and urinary tract; known more commonly as abbreviation CAKUT	Kidney aplasia/hypoplasia/dysplasia, vesicoureteral reflux, reflux nephropathy, obstructive uropathy anomalies (e.g. posterior urethral valves)
	Hereditary nephropathies	Polycystic kidney disease, Alport syndrome, nephronophthisis, congenital nephrotic syndrome
	Metabolic causes	Cystinosis, primary hyperoxaluria, methylmalonic acidemia
	Syndromic causes	Bardet-Biedl, Joubert, Branchio-oto-renal, Townes-Brocks syndrome
Acquired	Glomerular diseases	Focal segmental glomerulosclerosis, membranoproliferative glomerulonephritis, postinfectious nephropathy, IgA nephropathy
	Acquired obstructive reflux nephropathy	Urolithiasis, iatrogenic causes
	Haemolytic uremic syndrome	Most commonly due infestation with shiga toxin producing E. coli (STEC) and complement dysregulation; some hereditary causes also possible
	Interstitial nephritis	Infections, medications, autoimmune diseases (lupus nephritis)
	Infections	Recurrent pyelonephritis, tuberculosis
	Neoplasms	Wilms tumour most common; may also cause obstruction
	Drug-induced nephrotoxicity	Non-steroidal anti-inflammatory drugs, chemotherapy agents, calcineurin inhibitors, antibiotics (most commonly aminoglycosides), radiation nephritis

are lacking; however, some studies indicate that the prevalence of all-stage CKD among children may be as high as 1% (2,3). Furthermore, CKD in children presents numerous challenges in terms of its diagnosis, treatment, and long-term management. Several unique aspects have also been defined by KDIGO: growth, nutrition, weight/body-surface-area-based drug dosing, neurocognitive development, education support, transition to adult care, and holistic approach to care for the whole family unit (1). The impact of CKD on a child's physical, emotional, and cognitive development underscores the need for a multidisciplinary approach to care. Early intervention with prevention and individualized treatment plans can significantly improve quality of life and long-term outcomes of affected children.

AETIOLOGY

The aetiology of CKD in children varies by age. It includes congenital CKD, more likely diagnosed in infancy, and acquired causes, seen more commonly in later childhood and adolescence. Currently, an increasing number of patients are diagnosed antenatally (4). Congenital disorders are responsible for about two-thirds of all cases in the developed world, while acquired causes predominate in countries in development (5). A comprehensive list of congenital and acquired causes of paediatric CKD is presented in Table 2, with examples provided (5,6). Several diagnostic tools are used to establish the diagnosis, mainly imaging (ultrasound, magnetic resonance), specific laboratory markers, genetic testing, and kidney biopsy (7). In recent years, new management protocols for children with different kidney diseases potentially progressing to CKD have also been published, facilitating treatment of children in accordance with the latest guidelines (8,9).

DIAGNOSIS

After the aetiological determination, the stage of CDK is established based on GFR and albuminuria. In the case of the latter, first morning urine sample is preferred initially with protein/creatinine or albumin/creatinine ratio determination (1). Still, the key to determining the stage of CKD is the measurement or evaluation of GFR, critical also for monitoring

the progression of the disease. Estimating GFR using serum creatinine is the simplest tool, but in children it is not always informative due to changing muscle mass and growth. Nevertheless, serum creatinine measurement is inexpensive and a component of routine biochemical blood tests. Creatinine-based estimated GFR is therefore most widely used to obtain information on kidney function (10). Creatinine-based evaluation should include expected range of values for the age of the patient; also, estimated GFR should be determined with validated equations in children (1). Cystatin C has become an interesting alternative kidney biomarker, and recently, GFR estimation using equations with cystatin C has been gaining more attention (10). The most reliable method is GFR measurement, and for children, it is preferable to avoid use of ionizing agents. This method usually requires an intravenous injection of an exogenous filtration marker, such as iohexol, the use of which is becoming increasingly common, as well as several blood samples to determine the concentration-time decay curve, meaning that the method is burdensome and less appropriate in children (10). This and other studies are focused on simplifying the procedure with the same accuracy to provide a clinically useful method for GFR measurement (11). According to our research, this could be achieved by using only a 2- or 3-point sampling protocol (12).

In children under 2 years of age, the cut-offs from Table 1 do not apply, because they normally have a GFR lower than 60 ml/min/1.73 m2. Instead, the GFR is estimated and defined as mild kidney function impairment when it is decreased to more than one standard deviation from normal, moderate when decreased by two standard deviations, and severe when decreased by three standard deviations (13).

MANAGEMENT APPROACH BY STAGE OF CKD

Management of a child with CKD depends on its stage; however, some key components of management are incorporated in all-stage kidney disease: routine health maintenance with cardiovascular risk stratification, prevention or slowing the progression of kidney disease, preventing and treating the complications of CKD and preparation for kidney replacement therapy, if needed (1). Children with stages G1 and G2 are usually asymptomatic and are therefore harder to identify. They should be closely monitored due to hazard of kidney function deterioration (1,7). Children, who progress to stages G3a and G3b frequently begin to display CKD-associated complications – disorders of fluid and electrolyte balance, anaemia, hypertension, dyslipidaemia, endocrine abnormalities, growth impairment, mineral and bone disorder, and decreased clearance of substances (14). Patients who progress further to stage G4 should start preparations for kidney replacement therapy (1). Albuminuria and GFR should be assessed at least annually in children with CKD and further evaluated when a change in eGFR is >20% or when albumin/creatinine ratio is doubled (1).

ROUTINE HEALTH MAINTENANCE AND TREATMENT OF CKD-RELATED COMPLICATIONS

Critically, since CKD can affect child growth and development, meticulous attention should be paid to interventions aimed at growth and nutrition. Outpatient clinic and hospital visits should include height and weight measurements along with head circumference monitoring in children under 3 years of age. With the progression of paediatric CKD, appetite and nutritional intake decrease, resulting in malnutrition due to poor appetite, decreased intestinal absorption of nutrients, and metabolic acidosis associated with CDK, which affects overall health status. This condition is referred to as fragility phenotype in children with CKD (15). On the other hand, obesity is a risk factor for CKD, since it has the potential to accelerate deterioration of already existing CKD or cause CKD development through the interplay among obesity, insulin resistance, and renal hemodynamics (16). Therefore, it is recommended that children with CKD aim to maintain normal weight. When children do not meet the criteria for normal weight, a nutritional assessment should be carried

out. The initial prescribed energy intake for children with CKD is similar to that for healthy children of the same age. With advancing disease, recommended dietary intake is increased, preferably by oral route or by different feeding tubes, as necessary (1,17). Supplementation should be considered if the child's initial intake does not meet their estimated energy needs and they are not gaining weight or growing at the expected rate. Furthermore, protein restriction is not advised due to the risk of growth impairment and should be higher in patients on peritoneal dialysis due to dialytic protein loss (1,17). Adequate vitamin and mineral intake is equally essential (1).

Routine health maintenance also includes other aspects of general well-being, with special consideration given to cardiovascular risk factors (e.g., sedentary lifestyle, blood pressure, dyslipidaemia, under-/over-weight). Children with CKD should be physically active every day and are advised to maintain a healthy weight (1). Office blood pressure (BP) measurement should be performed at each healthcare visit. Strict BP control is essential to slow the progression of the kidney disease (18). In children with CKD, 24-hour mean arterial pressure by ambulatory BP monitoring should be lowered to ≤50th percentile for age, sex, and height (1,18). Patients with CKD and elevated BP should have echocardiogram as these patients are at risk for left ventricular hypertrophy, associated with adverse cardiovascular disease (19).

Laboratory testing is used to monitor kidney function and detect associated CKD complications; the frequency of assessment is based on the severity of kidney dysfunction. Commonly used tests include serum creatinine, urea, electrolytes with calcium and phosphorus, cystatin C, bicarbonate, alkaline phosphatase, albumin, haemoglobin, indices of iron status (ferritin, iron), fasting lipid profile, 25-hydroxyvitamin D, parathyroid hormone, urinalysis and urinary protein/creatinine ratio. With unexpected results or specific CKD aetiology,

Table 3: Complications of chronic kidney disease in children with their management and treatment (20).

Complication	Management and treatment
Nutrition disorders	Monitoring of anthropometric measurements, optimization of caloric intake, restriction of protein (rarely in children), phosphate, potassium, sodium and renal solutes in the diet according to needs, consideration of dietary supplements for appropriate weight gain, consideration of gastrostomy
Growth impairment	Ensure adequate nutrition, treat acidosis and sodium deficiency, consider growth hormone therapy, prevent/treat mineral bone disease
Chronic kidney disease-mineral and bone disorder	Ensure maintenance of adequate levels of calcium, phosphate, calcium and phosphate product, and parathyroid hormone; if plasma 25-OH vitamin D levels are low, supplement vitamin D3 (cholecalciferol 400–800 U/day); correct metabolic acidosis with sodium bicarbonate; consider vitamin D analogues (calcitriol) if calcium levels are low and parathyroid hormone levels exceed recommended levels; if plasma phosphate levels are high, restrict dietary phosphate, phosphate binders if needed
Neurodevelopmental delay, lower quality of life	Promoting school engagement, preventive counselling for parents and patients
Anaemia	Parenteral erythropoietin once to twice a week or darbepoetin once a week or every two weeks; iron supplementation orally 6 mg/kg/day two to three times a day, intravenously once a week at a maintenance dose of 2 mg/kg to a maximum of 100 mg; supplementation 7 mg/kg to a maximum of 200 mg in case of iron deficiency
Hypertension and cardiovascular disease	Drug therapy (mainly renin-aldosterone-angiotensin system blockers) to achieve normal blood pressure for age; limitation of calcium-containing phosphate binders and daily calcium intake to 2500 mg
Hyperlipidaemia	Lipid-lowering medications
Metabolic acidosis	Adding bicarbonate solutions
Hyponatremia	Adding salt, unless it is dilutional hyponatremia
Hyperkaliemia	Dietary potassium restriction, potassium exchange resins
Proteinuria	Renin-aldosterone-angiotensin system blockers, some calcium channel blockers, and beta-blockers
Hyperphosphatemia	Dietary phosphate restriction, phosphate binders

additional testing is required. Table 3 presents a summary of CKD complications and treatments (20). Targeted and early treatment of the specific aetiology of disease can slow or prevent progression to higher stages of CKD or even end-stage renal disease, optimizing the prognosis of renal outcome (7).

PREVENTION AND SLOWING THE PROGRESSION OF KIDNEY DISEASE

Some paediatric studies have confirmed that the time course of CKD development and progression can be variable and influenced by a number of potentially modifiable and non-modifiable risk factors (21,22). Reported interventions to slow CKD progression include BP control, reducing protein excretion, correcting anaemia, and maintaining normal 25-hydroxyvitamin levels (23,24). Avoiding acute episodes of kidney hypoperfusion and nephrotoxic drugs is of equal importance. Newer medications, such as SGLT2 (sodium-glucose transporter 2) inhibitors, are not yet approved for the treatment of CKD in children, though they have been shown to slow its progression in adult patients with both diabetic and non-diabetic CKD. Because they improve renal hemodynamic adaptation and provide additional beneficial effects on general complications of CKD, SGLT2 inhibitors are potential drugs for the treatment of CKD and glomerular diseases, including those in children (25).

Chronic kidney disease is a unique and challenging disease in childhood, the complications of which affect not only the child but also the person the child will grow up to be. Looking to the future of paediatric nephrology patients, we are aware that the leading cause of morbidity and mortality is cardiovascular risk. The common denominator is atherosclerosis, which is accelerated in these patients. Several newer functional (pulse wave velocity) and imaging techniques (intima media thickness, ultrasound elastography) and biochemical markers (salusins, adropine, kidney-injury molecule 1, inflammatory markers, miRNA ...) are being researched to facilitate accurate assessment of cardiovascular risk in children with CKD and timely intervention (26–29).

NOVEL THERAPIES FOR CKD IN ADULTS AND CHILDREN

A range of novel therapies for CKD are emerging, primarily in the adult population, with promising potential for application in paediatric care. We emphasize the critical role of basic, translational, and clinical research in advancing this field, along with the need to better define combination therapies that target multiple disease pathways.

In addition to SGLT2 inhibitors, finerenone – a non-steroidal mineralocorticoid receptor antagonist with anti-inflammatory and anti-fibrotic properties – is gaining traction. It has demonstrated cardio-renal benefits in adults with type 2 diabetes mellitus (30). Ongoing studies are currently exploring its use in children with CKD and proteinuria (31). For paediatric patients with obesity, growing evidence suggests the possible therapeutic role of glucagon-like peptide-1 receptor (GLP-1R) agonists (32).

Inherited kidney diseases are a significant cause of CKD in children, posing a lifelong burden. Exciting progress is being made in treatment strategies, particularly in the realm of genetic therapies, which are rapidly advancing. Gene therapy is being investigated in animal models for conditions such as Alport syndrome, Dent disease, Fabry disease, primary hyperoxaluria, and cystinosis, with some studies progressing to preclinical stages, e.g. in both autosomal dominant and recessive polycystic kidney diseases (33).

Other therapeutic agents under investigation include microRNAs for Alport syndrome, PCSK9 inhibitors and sparsentan for congenital nephrotic syndrome, and calcineurin inhibitors and vaptans for polycystic kidney diseases. For tubulopathies, drugs such as indomethacin, acetazolamide, spironolactone, cyclooxygenase inhibitors, and hydrochlorothiazide are being explored. In Fabry disease, lucerastat, pegunigalsidase-alfa, and adeno-associated virus vectors are under study. Treatments for primary hyperoxaluria include RNA interference-based therapies such as Lumasiran and Nedosiran, as well as stiripentol, while cysteamine bitartrate continues to be evaluated and used for cystinosis (34, 34).

More recently, the therapeutic landscape has seen a surge in agents targeting complement system inhibition. Promising data have emerged for agents such as eculizumab, ravulizumab, crovalimab, avacopan, danicopan, iptacopan, pegcetacoplan, and narsoplimab. These therapies have shown effectiveness in reducing proteinuria and stabilizing kidney function in various complement-mediated kidney disorders. Given their high efficacy and target specificity, these drugs hold the potential to significantly improve outcomes in affected children (35).

Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs), already established in the management of hypertension, proteinuria, and CKD progression, continue to be investigated in paediatric populations. Current research is focused on evaluating whether combined ACEI and ARB therapy offers additive benefits and improved clinical outcomes (36).

Recent advancements in paediatric renal replacement therapy (RRT) have led to the development of specialized devices tailored for neonates and infants, addressing the limitations of adapting adult-sized equipment for small patients, and offering precise fluid management and improved safety profiles. These technologies have demonstrated effectiveness in managing acute kidney injury and fluid overload in critically ill newborns, expanding the therapeutic options available for this vulnerable population (37). Finally, novel palliative interventions for children with advanced CKD involve life participation and care plan development, allowing for holistic and family-centred management (38).

CONCLUSIONS

In summary, treating children with CKD involves promoting a healthy lifestyle, slowing disease progression, managing complications, and preparing for potential renal replacement therapy. This requires regular monitoring, addressing underlying causes, preventing further kidney damage, and ensuring proper nutrition, blood pressure, and proteinuria control. Care should be multidisciplinary and family-centred, starting early to support the child's overall well-being and quality of life.

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Uporaba nanotomografije v biomedicinski znanosti in medicini

The application of computed nanotomography in biomedical sciences and medicine

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Izvleček

Namen: *Računalniška nanotomografija (nano-CT) je napredna slikovna tehnika, ki omogoča visoko ločljivo tridimenzionalno (3D) vizualizacijo na celični in subcelični ravni, ter tako bistveno presega zmogljivosti tradicionalnih tehnik računalniške tomografije (CT) in drugih slikovnih metod. Ta pregledni članek obravnava tehnološke novosti nano-CT, njegove trenutne zmogljivosti in potencialno vključitev v klinično medicino.*

Metode: *Izvedli smo pregled novejše literature in študij s področja tehnologije nano-CT, s poudarkom na njegovi uporabi v biomedicinskih raziskavah ter potencialni integraciji v klinično prakso.*

Rezultati: *Nano-CT se je izkazal kot zelo uporabna tehnika v različnih vejah medicine, vključno*

Abstract

Background: *Computed nanotomography (nano-CT) is a cutting-edge imaging technology that provides ultra-high-resolution three-dimensional (3D) visualization at the cellular and subcellular levels, significantly surpassing conventional computed tomography (CT) and other imaging modalities. In this review, we describe the technological development of nano-CT along with its current capabilities and potential for integration into clinical medicine.*

Methods: *We reviewed the existing literature relating to nano-CT technology, emphasizing biomedical research applications and evaluating potential for future clinical integration.*

Results: *Nano-CT has demonstrated exceptional utility across various*

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s kardiologijo, vaskularno medicino, nevrologijo, pulmologijo, onkologijo in zobozdravstvom. Omogoča edinstven vpogled v arhitekturo tkiv, celične podrobnosti in patološke procese, kar bistveno izboljšuje razumevanje bolezni in podpira napredek v diagnostiki, načrtovanju zdravljenja ter novih terapevtskih strategijah.

Zaključki: *Kljub izjemnemu potencialu na področju biomedicinskih raziskav je klinična uporaba nano-CT še vedno soočena z več izzivi, kot so omejitve v hitrosti slikanja, kontrastu mehkih tkiv, sevalni obremenitvi in velikosti vzorcev. Nadaljnje tehnološke inovacije, validacija s kliničnimi študijami ter razvoj multimodalnih slikovnih pristopov so nujni za uspešno integracijo nano-CT v rutinsko medicinsko diagnostiko in personalizirano medicino.*

biomedical disciplines, including cardiology, vascular medicine, neurology, pulmonology, oncology and dentistry. This imaging technique provides unprecedented insights into tissue architecture, cellular details and pathological processes, providing significant enhancement in our understanding of diseases and supporting advancements in diagnosis, treatment planning, and therapeutic strategies.

Conclusions: *Despite remarkable potential for biomedical research, the clinical adoption of nano-CT faces several challenges, including limitations in imaging speed, soft tissue contrast, radiation dose and sample size. Continued technological innovations, clinical validation, and the development of multimodal imaging approaches are essential for the successful transition of nano-CT into routine medical diagnostics and personalized medicine.*

INTRODUCTION

Despite the accelerated technological development of X-ray-based computed tomography (CT) over recent years, especially with regards to temporal resolution; the spatial resolution of this cross-sectional imaging method in the clinical setting still lies between 500 µm and 625 µm (1). Spatial resolution in CT depends on several factors, such as X-ray focal spot size, the number of projection views per X-ray tube rotation, the size of the detector cell, and reconstruction algorithms (1). Recent advances in detector technology, iterative reconstruction algorithms, and X-ray source optimization have improved resolution capabilities, although fundamental limitations remain due to the physical properties of X-ray sources and detector elements (2-4). The first in vivo scanners with a spatial resolution of 105 to 55 µm (high-resolution peripheral quantitative CT scanners, HR-pQCTs) are already being used to image microscopic bone structures (5, 6). Structures smaller than this resolution cannot be imaged with this CT technology. To image structures smaller than 200 µm, histological and pathohistological techniques still remain the gold standard. However, histological methods have their own disadvantages, including labor-intensive sample preparation, irreversible tissue destruction, and potential artifacts introduced during processing (7). Computed microtomography (micro-CT) is a well-established complementary technique to histological examinations as a non-destructive three-dimensional (3D) imaging technique with micrometer resolution (8). The non-destructive nature of the CT technique is one of the most important advantages over histology. Tissues used as examination specimens in micro-CT devices can subsequently be used as samples for further pathohistological studies after the examination has been completed.

There is no uniform definition of micro-CT in the existing literature. Kalender (2011) suggested a threshold spatial resolution of at least 100 µm and reported that CT devices with better spatial resolution can be referred to as micro-CT devices, irrespective of other device specificities (9). Micro-CT systems, typically achieving spatial resolutions between 5 and 50 µm, provide essential insights

into the 3D microarchitecture of various biological tissues, bridging the gap between clinical imaging and microscopy. This technique is gradually creating opportunities for virtual histology, live cell imaging, subcellular imaging, and correlative microscopy (10). This combination of histology and radiological techniques was first applied to study the morphology of spongy bone (6). Over recent years, with the development of radiocontrast agents, micro-CT has been applied in many other fields, such as [1] the visualization and quantification of pathological vascular lesions in animal models (8); [2] generating pulmonary and cardiac function parameters from animal models (8) and [3] the evaluation of local biomechanical behavior under complex loading conditions (11, 12). Nevertheless, micro-CT, when performed both in vivo and ex vivo) is still limited by spatial resolution. This limitation is particularly evident when imaging structures such as terminal vessels, the components of arteriosclerotic plaques, and cellular lacunes in calcified hard connective tissue, which represent structures that are too small to discern with the use of micro-CT devices (8).

Building upon the advancements of micro-CT, computed nanotomography (nano-CT) has emerged as a cutting-edge imaging modality that offers sub-micrometer spatial resolution that significantly surpasses the capabilities of micro-CT. Nano-CT systems employ specialized transmission-target X-ray tubes featuring a focal spot size < 400 nanometers, achieving spatial resolutions down to approximately 50–400 nm, thus enabling detailed visualization of structures at the cellular and even subcellular level (8). One of the primary advantages of nano-CT is its ability to provide non-destructive and 3D imaging of biological specimens at the cellular level in a manner that preserves the integrity of samples for subsequent analyses. This feature is particularly beneficial for certain fields, such as pathology, for which traditional methods require labor-intensive sample preparation and result in tissue destruction. Nano-CT facilitates virtual histology, allowing for the detailed examination of tissue architecture without physical sectioning (13-18).

In the realm of vascular imaging, nano-CT has demonstrated exceptional utility by revealing microvascular networks and intricate plaque compositions in atherosclerotic models. The high spatial resolution of this method enables the detection of intraplaque hemorrhages and calcifications that are not discernible with micro-CT, thereby enhancing our understanding of vascular pathologies (19). Moreover, nano-CT has proven invaluable in dental research, particularly in evaluating root canal morphology and the quality of endodontic treatments. The ultra-high resolution of nano-CT allows for the clear visualization of complex root canal systems and the assessment of treatment efficacy, thus providing insights that are critical for improving dental procedures (20, 21).

Despite its numerous advantages, nano-CT is primarily limited to ex vivo applications due to both technical and physical constraints. The high radiation doses, prolonged scanning times, and limited field-of-view associated with nano-CT imaging currently restrict its application in vivo. However, recent advancements, such as phase-contrast imaging and improved detector efficiency, are gradually reducing both radiation exposure and scan duration, thus enabling limited in vivo applications, particularly for research involving small animals (22). It is important to note that nano-CT necessitates the use of contrast agents to enhance the visualization of soft tissue, as soft tissues inherently exhibit low X-ray absorption (8). Thus, nano-CT represents a significant step forward in imaging technology, offering a better resolution that bridges the gap between traditional micro-CT and histological methods. Furthermore, the ability of nano-CT to visualize biological structures at the cellular level in a non-destructive manner holds significant promise for the advancement of research in various medical fields, including histopathology, vascular medicine, and dentistry.

THE NANO-CT TECHNIQUE

Nano-CT, often referred to as nanotomography, is an advanced imaging technique that enables 3D visualization of objects at the nanometer scale. Building upon the principles of traditional CT, nano-CT employs highly focused X-ray beams and

sophisticated detectors to achieve superior spatial resolution, often reaching sub-400-nanometer levels. This exceptional spatial resolution is primarily attributed to the use of specialized transmission-target X-ray tubes that possess focal spot sizes < 400 nanometers, as well as advanced detector technology and precise sample positioning systems, significantly surpassing the capability of conventional micro-CT systems (8, 23).

Nano-CT imaging involves rotating the specimen and capturing multiple two-dimensional (2D radiographic images from various angles. These images are then reconstructed computationally to produce a detailed 3D representation of the internal structure of a given specimen. The reconstruction algorithms, including iterative and filtered back-projection methods, play a crucial role in achieving high-quality volumetric data from the acquired projections. This non-destructive technique is invaluable when investigating the intricate architecture of biological tissues, materials, and chemical compounds without altering their inherent properties (23, 24).

Recent advancements in nano-CT technology have expanded the application of this technique across various scientific domains. For instance, the development of stress nanotomography allows us to map internal structures and stress distributions in materials at the nanoscale, achieving resolutions approximately 100-fold higher than traditional X-rays and neutron tomography (25, 26). Furthermore, developments in phase-contrast imaging and synchrotron-based nano-CT have significantly improved contrast resolution, particularly in soft tissues and materials with low absorption differences, thus broadening the applicability of nano-CT to a diverse range of scientific disciplines (27, 28). This innovation holds significant promise for nanotechnology and materials science as it could provide deeper insights into material properties and behaviors (23, 29).

In the field of medicine, nano-CT offers unprecedented opportunities for the detailed visualization of biological specimens. The high-resolution imaging capabilities of nano-CT facilitate the investigation of cellular and subcellular structures and can contribute to a better understanding of disease mechanisms and the development of targeted therapies. Furthermore, nano-CT holds promise for detailed investigations of pathological processes such as tumor angiogenesis, microcalcifications in cancerous tissues, and neurovascular remodeling in neurological diseases, thereby facilitating the discovery of novel biomarkers and therapeutic targets (8, 30-32). As nano-CT technology continues to evolve, this method is poised to become an integral tool in both research and clinical settings to enhance our ability to diagnose and treat various medical conditions with greater levels of precision (23).

General aspects and working principles of nano-CT

In many languages, X-radiation is referred to as Röntgen radiation, after the German scientist Wilhelm Conrad Röntgen after his initial discovery in 1895 (33). X-rays are produced in a vacuum tube that contains two main electrodes: a cathode and an anode, positioned at opposite ends of the tube. The cathode typically consists of a coiled filament, while the anode is located directly across from the cathode. In conventional X-ray machines, the anode is usually made of copper, with a tungsten target at the focal area that can withstand high temperatures. When the cathode filament is heated, typically by a low firing voltage of 8–12 V, it emits electrons by thermionic emission. These electrons are then accelerated towards the anode by a high direct current (DC) voltage ranging from 10 to 150 kV, thus creating the conditions necessary to generate X-rays (34-36). The accelerated electrons, also known as cathode rays, strike a small, designated area of the anode known as the focal spot. Upon impact, these electrons are rapidly decelerated, resulting in the release of energy. The majority of this energy, approximately 99%, is converted into heat, while only approximately 1% is emitted as electromagnetic radiation in the form of braking radiation, commonly known as X-rays (35, 36). Figure 1 depicts how X-rays are formed.

The resulting narrowly confined beam of X-ray photons, which is rotated around the subject/patient in conventional CT machines in clinical practice,

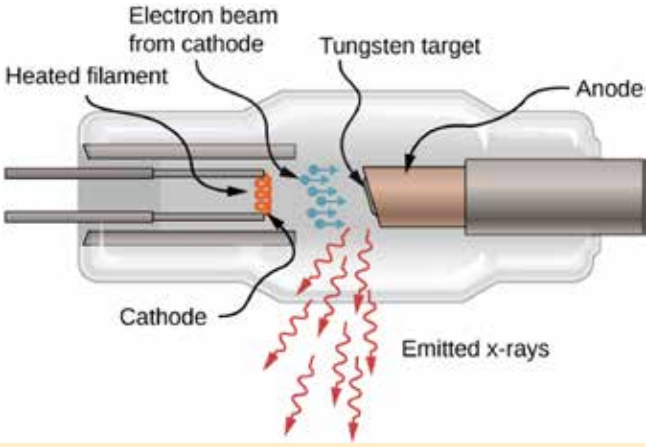

Figure 1. *A schematic showing the formation of X-rays with the most important components annotated. LibreTexts. Licensed under CC BY-NC-SA 3.0. Photo via Fotoeins Fotografie: https://fotoeins.com/2019/06/10/wuerzburg-roentgen-memorial-xrays/xrays-libretexts/*

travels towards the object and attenuates as it passes through the object. The light passed through an object is measured by a system of detectors, and the signals derived from the individual detectors are assembled into a profile (projection). These projections originate from various angles and undergo computational reconstruction, typically via filtered back-projection or iterative reconstruction algorithms, to generate a cross-sectional image of the specimen or patient. Each spatial element in the reconstruction is assigned an attenuation coefficient (μ), which is specific to the density and atomic composition of the substance. Instead of the raw attenuation coefficient, CT imaging utilizes relative values compared to water, known as Hounsfield Units (HU). This scale is named after Sir Godfrey Hounsfield, inventor of the X-ray CT scanner (37). Thus, each pixel in a final CT image represents the two-dimensional projection of the X-ray attenuation from a voxel (volume element) within the observed object (37).

Nano-CT originates from the further development of micro-CT technology and has achieved superior resolution via the use of transmission-target X-ray tubes with significantly smaller focal spots (< 400 nm), advanced high-resolution detector arrays, precise geometric magnification, optimized angular scanning protocols, and noise reduction techniques that involve repeated imaging sequences (8). These enhancements allow imaging at the sub-micron scale, hence the term nano-CT, coined specifically to differentiate these advanced systems from micro-CT technology. The unprecedented ability of nano-CT to detect detailed subcellular features can allow researchers to investigate biological questions that were previously beyond the capabilities of conventional micro-CT, thus exerting significant impact on fields such as histology, developmental biology and materials science.

Development of nano-CT and differences between nano-CT and other CT technologies

Until the late 1980s, CT scanners operated by a process known as axial scanning in which data were acquired sequentially in discrete slices. However, newer techniques have been developed, most prominently spiral or helical CT, in which the observed object translates through a gantry while the scanner rotates continuously around the object. This allows for new options in reconstruction. Spiral CT provides significant advantages compared to axial CT, particularly in terms of minimizing motion artifacts, reducing radiation exposure essential for in vivo imaging, improving spatial resolution along the Z-axis, and enhancing 3D image rendering capabilities (37).

During the early 1980s, the first micro-CT scanner was developed for the automotive industry by physicist Lee Feldkamp from Ford Motor Company (11). In 1984, Steven Goldstein from the University of Michigan replicated the micro-CT system in his own laboratory, establishing the first university micro-CT system that was built to allow the investigation of biomechanics (11).

High-resolution CT at the sub-micron level has primarily been considered the domain of synchrotron-based micro-CT (synMCT) (8). This technology is regarded as the gold standard for sub-micron imaging due to its exceptionally high photon flux, monochromatic beam capabilities, and enhanced

phase-contrast imaging performance, which can significantly increase image contrast and resolution when compared to laboratory-based CT systems. Unfortunately, synMCT is not a stand-alone device but rather a method that is implemented in large synchrotron centers, such as "The German Electron Synchrotron" (DESY), the "Conseil Européen pour la Recherche Nucléaire" (CERN) and "The National Synchrotron Light Source" (NSLS). Conversely, nano-CT systems represent stand-alone high-resolution devices that are designed for laboratory use and provide comparable resolutions but significantly greater accessibility and convenience when compared to synchrotron-based methods (8).

One primary operational difference between conventional medical CT and nano-CT is that in nano-CT systems, the specimen rotates around its own axis rather than the X-ray source and detectors rotating around the specimen. Once the sample is illuminated, the X-rays can reach the detector (8). X-ray absorption and diffraction patterns in the sample provide detailed structural information that are critical for high-resolution imaging. Unlike the microfocus X-ray tubes (reflection/direct-beam tubes) used in micro-CT, nano-CT systems utilize transmission tubes in which the X-ray window and focal spot coincide. This configuration allows the specimen to be positioned much closer to the focal spot, substantially reducing the effective focal spot size to approximately 500 nm and significantly enhancing achievable spatial resolution, often down to the sub-micron range (8).

A comparison of nano-CT and other imaging techniques in medicine: advantages and limitations

Advantages and limitations of imagine techniques in general

Computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) are all widely used imaging techniques in medicine, each with its own strengths and limitations. CT scanning, and PET scanning that uses 18F-fluorodeoxyglucose, involve ionizing radiation, which presents a critical consideration for repeated in vivo studies, especially in pediatric and longitudinal research contexts (38). MRI, on the other hand, has a key advantage in that it does not involve ionizing radiation.

CT is valued for its rapid acquisition times (39), making it ideal in emergency settings, such as the assessment of trauma or stroke. MRI and PET generally have longer acquisition times, which can cause discomfort (39) and anxiety in patients with claustrophobia, often necessitating sedation for MRI studies (40, 41). CT excels in providing high levels of spatial resolution; this is particularly useful for detecting small lesions, and provides excellent bone contrast, thus allowing detailed evaluation of skeletal structures. However, CT is not without its drawbacks. The primary disadvantage of this technique is exposure to ionizing radiation, raising concerns relating to cumulative radiation doses, particularly in pediatric populations or in patients requiring repeated scans. Furthermore, CT involves limited soft tissue contrast, often requiring the use of contrast agents to enhance the visualization of organs and vascular structures.

MRI, on the other hand, provides superior soft tissue contrast, making it particularly useful for brain, musculoskeletal and abdominal imaging (39). Furthermore, MRI offers multi-parametric capabilities, including diffusion-weighted imaging (DWI), functional MRI (fMRI), and spectroscopy, enabling comprehensive structural and functional evaluations. A major advantage of MRI is the absence of ionizing radiation (40), rendering this technique a safer option for repeated imaging, particularly for young patients. Moreover, the contrast material used for MRI is safer for the kidneys than that used for CT (40). In addition, MRI has certain limitations, such as longer acquisition times, that can be problematic for critically ill or restless patients. Also, the loud noises generated during scanning necessitate the use of hearing protection (40). Another consideration is that MRI is more expensive and less widely available than CT, and certain patients, particularly those with metal implants such as pacemakers, may not be suitable for MRI scans.

PET imaging, frequently combined with CT or

MRI (as in PET/CT or PET/MRI), is invaluable for functional imaging, especially in the fields of oncology, neurology and cardiology (39). The high sensitivity of PET to metabolic and molecular activity enables the early detection of disease and the precise assessment of physiological processes such as glucose metabolism and cerebral blood flow. Nevertheless, PET imaging is generally associated with lower spatial resolution when compared to CT or MRI (39), thus limiting its anatomical localization capabilities. PET involves exposure to radiation; although this is typically lower than the dose used for diagnostic CT scans (38, 42), the dose of radiation used in PET can vary significantly based on scan protocols, resolution requirements, and the specific type of radiotracer used (42). PET is also associated with high costs and logistical challenges due to the need for specialized radiotracers with short half-lives. In addition, acquisition times are generally longer than in CT (39).

In conclusion, while CT, MRI, and PET each offer unique strengths, they also present specific limitations that dictate their suitability in clinical and research contexts. The selection of an appropriate imaging modality depends critically on the clinical or research question, the requirement for anatomical or functional data, radiation safety considerations, and individual patient factors such as age, health status and the presence of implants.

A comparison of high-resolution 3D imaging techniques

High-resolution 3D imaging plays a crucial role in medical diagnostics and research as it can allow the detailed visualization of anatomical structures and physiological processes. Although CT, MRI, and PET all provide 3D imaging capabilities, these methods differ significantly with regards to spatial resolution, contrast sensitivity, acquisition speed, functional assessment capabilities, and levels of radiation exposure (Table 1).

CT, MRI, and PET each offer distinct advantages and limitations with regards to high-resolution imaging tailored to specific clinical or research requirements. CT, notably in micro-CT and nano-CT formats, delivers superior high-resolution 3D structural imaging, with nano-CT achieving spatial resolutions down to sub-400 nm levels. This high spatial resolution, combined with rapid acquisition times, makes CT particularly suited for the detailed visualization of hard tissues, such as bone and calcified structures. Nevertheless, CT is associated with significant limitations, including comparatively poor intrinsic soft tissue contrast, necessitating the use of contrast agents for adequate soft tissue differentiation, and exposure to ionizing radiation, which limits its frequent in vivo use, especially in longitudinal studies.

MRI is renowned for its exceptional soft tissue contrast and provides detailed 3D anatomical images without the need for ionizing radiation, rendering this technique particularly advantageous for repeated longitudinal imaging studies and safer for application in pediatric patients. MRI also supports advanced multi-parametric imaging modalities, such as functional MRI (fMRI), diffusion-weighted imaging (DWI), and spectroscopy, enabling

Table 1: Comparison of computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET). fMRI – functional MRI; DWI – diffusion-weighted imaging.

Modality	Spatial Resolution	Soft Tissue Contrast	Functional Imaging	Radiation Exposure	Acquisition Time
CT (micro/nano)	High (50–100 µm for micro-CT, sub-400 nm for nano-CT)	Moderate (improved with contrast agents)	No	Yes	Fast
MRI	Moderate to High (50–100 µm for high-field MRI)	Excellent	No (except fMRI, DWI)	No	Long
PET	Low (1–2 mm)	Poor (relies on CT/MRI fusion)	Yes	Yes	Long

both structural and functional assessments at relatively high resolutions. However, MRI typically achieves spatial resolutions in the micrometer range that are lower than those of nano-CT. The limitations of MRI include prolonged scan durations, susceptibility to motion artifacts (which are problematic for non-compliant or critically ill patients), high operating costs, and the limited accessibility of high-field MRI equipment.

PET imaging primarily provides functional information rather than detailed anatomical structures. This method exhibits high levels of sensitivity for metabolic and physiological processes at the molecular level and is therefore invaluable for the detection of disease in the early stages, especially in oncology and neurology. The combination of PET with CT or MRI (PET/CT, PET/MRI) can significantly improve anatomical localization by integrating functional and structural data. However, PET imaging typically exhibits lower spatial resolutions (approximately 1–2 mm) compared to CT and MRI. Furthermore, PET involves radiation exposure from radiotracers, often requiring short half-life radiopharmaceuticals; this introduces logistical complexity, higher costs, and practical limitations.

In summary, for high-resolution 3D anatomical imaging, nano-CT provides the highest achievable spatial resolution, and is particularly beneficial for ex vivo research settings. MRI excels in soft tissue differentiation, yet its spatial resolution remains limited compared to nano-CT. PET offers indispensable functional imaging capabilities but lacks the spatial resolution necessary for fine structural detail. Hybrid imaging modalities (PET/CT, PET/MRI) effectively integrate complementary functional and anatomical information, overcoming the inherent limitations of standalone modalities and significantly enhancing diagnostic and research capabilities in both clinical and preclinical settings.

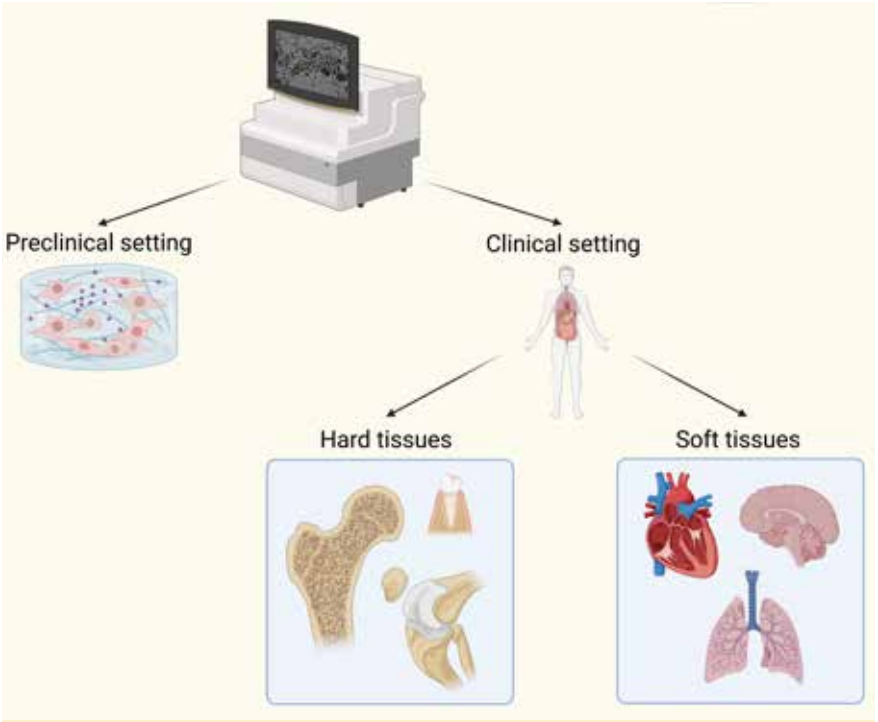

Figure 2. *Schematic showing the potential uses of nano-CT in both preclinical and clinical settings.*

SPECIFIC APPLICATIONS

Figure 2 depicts the potential applications of computed nanotomography (nano-CT) in the field of medicine.

Preclinical setting: research

Nano-CT has emerged as a powerful tool in preclinical and biomedical research as it provides ultra-high-resolution 3D imaging at the nanometer scale (50–400 nm). While conventional clinical CT is routinely used for diagnostic purposes, nano-CT is predominantly employed for ex vivo and studies due to current limitations. Nano-CT enables researchers to investigate intricate structural details in biological tissues, disease models, and biomaterials, and can provide precise volumetric and structural measurements. A key advantage of this technique is

its non-destructive nature, which preserves sample integrity for subsequent analytical methods (8).

Biomaterials and tissue engineering

Nano-CT plays a crucial role in the evaluation of biomaterial properties and their interaction with biological tissues. This technique can facilitate the detailed characterization of biomaterial scaffolds by evaluating a range of microstructural features, including porosity, pore interconnectivity, degradation patterns, and cellular infiltration within engineered tissues (43-48). Imaging quality can be further enhanced by contrast-enhanced nano-CT which is particularly beneficial for visualizing soft tissues. This technology can make significant contributions to nanoparticle distribution studies within drug delivery systems and enables the high-resolution visualization of pathological alterations in models of experimental disease (49).

Imaging is primarily conducted ex vivo, with limited in vivo applications constrained by prolonged scan times, radiation dose concerns, and restricted sample sizes. Soft tissue visualization typically necessitates the use of contrast agents. However, advances in phase-contrast imaging, synchrotron-based nano-CT, and multi-modal imaging approaches are poised to significantly enhance the capabilities of nano-CT. With continued improvements in acquisition speeds, reduced radiation doses, and the performance of soft tissue imaging, nano-CT is expected to increasingly bridge preclinical research and clinical translational applications.

Clinical settings

While nano-CT is currently predominantly utilized in preclinical research, recent technological advancements suggest promising potential for future clinical applications. Currently, nano-CT remains largely unimplemented in routine clinical care due to a number of constraints, including a small field-of-view, extensive scan durations, and concerns relating to radiation exposure. However, as technology progresses, the high-resolution 3D imaging capability of nano-CT may exert profound impact on diagnostics, surgical planning, and personalized medicine. Despite promising prospects, nano-CT is not yet applicable for routine clinical practice due to radiation safety issues, prolonged imaging times, and the necessity for small sample sizes. Nevertheless, future developments in phase-contrast techniques, synchrotron nano-CT methodologies, and artificial intelligence (AI)-driven image reconstruction algorithms could overcome these limitations, potentially facilitating the integration of nano-CT into precision medicine workflows (50, 51).

Hard tissues

Nano-CT provides an ultra-high-resolution and non-destructive imaging modality for bone and dental tissues. Compared to conventional micro-CT and clinical CT, nano-CT delivers sub-micrometer resolution, enabling detailed visualization of bone microarchitecture, dental structures and implant integration (6, 16, 50). In addition, nano-CT facilitates high-resolution 3D imaging of trabecular and cortical bone, surpassing micro-CT with regards to the detection of microscopic bone porosities and mineralization patterns (2). Furthermore, nano-CT provides detailed structural insights into fracture healing processes, including callus formation and bone remodeling in complex fractures. Nano-CT has also proved to be invaluable for the early detection of trabecular bone loss in osteoporosis and in the analysis of microstructural changes associated with metabolic bone diseases and rare skeletal disorders. Furthermore, nano-CT can significantly enhance the evaluation of bone metastases, tumor invasion into osseous structures, and implant osseointegration, thus providing detailed insights into bone-implant contact and peri-implant bone density. These capabilities are instrumental in optimizing orthopedic implants and investigating the mechanisms that underlie implant wear and failure (16, 52).

Nano-CT represents a significant advancement in the visualization of mineralized structures in dentistry and oral surgery, and can achieve levels of detail that are unattainable by conventional radiography. The sub-micrometer imaging capability of nano-CT enables researchers to investigate early-stage caries, enamel demineralization, and microfractures prior to clinical manifestation to provide insights into dental

tissue aging, wear, and erosion processes (26). Nano-CT also enhances the 3D visualization of root canal morphology and can elucidate complex variations in root systems (12). In addition, nano-CT can facilitate the assessment of endodontic therapy, evaluate the adaption of filling materials, periapical healing, and the osseointegration of dental implants. This imaging tool could also facilitate the development of biocompatible dental prosthetics, restorative materials, and veneers, and permit the investigation of adhesion properties and material degradation under realistic conditions (20, 53).

Challenges and future perspectives in the use of nano-CT for the imaging of hard tissue

Current nano-CT systems are unsuitable for live human imaging, thus restricting its application primarily to ex vivo analyses. The high-resolution imaging capability of this technique is constrained by narrow fields-of- view, limiting applicability to whole-bone or large joint evaluations. However, despite these limitations, nano-CT offers unparalleled resolution in hard tissue imaging, proving invaluable for bone pathology research, dental diagnostics, and biomaterials assessments. Ongoing technological advancements are expected to broaden the applications of nano-CT in the fields of clinical diagnostics and personalized medicine via faster imaging protocols, AI-enhanced reconstruction techniques are improving our ability to visualize soft tissue and facilitating integration with conventional clinical imaging methods such as CT and MRI. These developments will likely facilitate a comprehensive, multi-resolution approach to diagnostic evaluations and therapeutic planning in the future (50).

Soft tissues

Thus far, nano-CT has proved to be more effective for the imaging of hard tissue due to the high X-ray absorption properties of bone and dental structures. However, recent developments in contrast-enhancement methods, phase-contrast imaging, and synchrotron-based nano-CT have markedly improved the capability of nano-CT to visualize soft tissues at nanometer-scale resolution (54-56). Nano-CT is increasingly being integrated into histopathological workflows to enhance tissue analysis. This integration provides comprehensive 3D histological insights without the necessity for the sectioning of physical tissue, thereby preserving sample integrity and facilitating subsequent specialized analyses. These advancements open promising opportunities for clinical applications across various medical disciplines, including histopathology, oncology, neurology, cardiovascular medicine, and organ-specific research (50).

Nano-CT could represent a significant contributor to oncology research by facilitating the detailed assessment of tumor microarchitecture, vascularization, tumor-stroma interactions, and cellular heterogeneity. The ultra-high-resolution imaging capabilities of nano-CT enable the precise visualization of microstructural tumor characteristics, thus facilitating the early detection of disease, prognostic assessment, and personalized therapeutic planning (31). In addition, nano-CT facilitates the highly detailed imaging of microvascular networks in a manner that surpasses the capabilities of conventional 2D histology and other imaging modalities. This makes nano-CT particularly beneficial for applications in cardiovascular and vascular medicine. Potential applications include the comprehensive analysis of atherosclerotic plaque composition, microvascular remodeling within arterial tissues, the three-dimensional visualization of capillary networks, the detailed assessment of stent and vascular graft integration, and the characterization of heart tissue, including myocardial fibrosis and damage assessment in ischemic heart disease (8, 57).

Nano-CT can significantly enhance the evaluation of lung tissue architecture in pulmonary diseases such as chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, and lung damage related to COVID-19 infection. Furthermore, this technique allows for the precise characterization of alveolar structures, microarchitectural changes, collagen deposition, lung tissue remodeling, and inflammation-associated vascular alterations, thus facilitating detailed investigations into disease progression and pathophysiological mechanisms (8, 15, 58).

Another consideration is that nano-CT offers considerable potential for the field of neuropathology due to its ability to resolve subcellular features within neural tissues. This technique could significantly enhance research, diagnosis, and therapeutic approaches for neurodegenerative disorders by enabling detailed analyses of the microstructural alterations associated with Alzheimer's disease, Parkinson's disease, and multiple sclerosis. Moreover, nano-CT can facilitate the precise examination of brain microvasculature, synaptic architecture, and neuronal structures, providing deeper insights into the pathology of neurological disease (8, 59, 60).

Challenges and future perspectives in the application of nano-CT for soft tissue imaging

Soft tissues inherently exhibit low X-ray contrast; this poses challenges for nano-CT imaging. However, several strategies are being explored to enhance soft tissue visualization. First, contrast agents, such as heavy-metal-based staining agents (e.g., iodine, osmium and phosphotungstic acid) could improve detail at the cellular-level by increasing X-ray contrast. Second, phase-contrast nano-CT is under development; this technique aims to exploit variations in X-ray phase shifts to achieve label-free, high-contrast imaging of soft tissues. Third, synchrotron radiation-based nano-CT is under development. This technique aims to utilize the highly intense and coherent X-rays available at specialized synchrotron facilities to deliver superior soft tissue contrast and higher spatial resolution.

Although nano-CT is currently limited to ex vivo imaging for the analysis of clinical soft tissue, the application of this technique is rapidly expanding within the fields of histopathology, cardiovascular research, neurology and pulmonology. Ongoing advancements in contrast-enhancement techniques, multi-modal imaging integrations that combine nano-CT with functional imaging modalities (MRI, PET), along with further technological improvements, are anticipated to significantly broaden the clinical utility of nano-CT in the future.

CONCLUSIONS

Nano-CT represents a significant advancement in 3D imaging technology, providing unprecedented resolution at both the cellular and subcellular levels. The ability of this method to visualize intricate biological structures in a non-destructive manner has transformative implications for preclinical research, particularly in tissue engineering, the evaluation of biomaterials, and detailed pathological analyses across numerous medical fields, including oncology, cardiovascular medicine, pulmonology, neurology, and dentistry. Despite its evidential strengths and potential for significant clinical impact, the routine application of nano-CT in clinical settings remains limited due to several technological and practical constraints. These limitations include prolonged scan times, limited fields-of-view, concerns related to the dose of radiation, and challenges associated with soft tissue contrast. Nevertheless, ongoing technological innovations, particularly advancements in phase-contrast imaging, contrast agent development, synchrotron-based imaging techniques, and AI-driven reconstruction methods, are steadily overcoming these limitations. Future research and clinical validation are essential to fully realize the integration of nano-CT into routine diagnostic and treatment planning workflows, ultimately supporting precision medicine and personalized therapeutic strategies.

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Histološke prednosti dvo-komponentne fibrinske mreže (TachoSil) v primerjavi s polipropilensko mrežo po vstavitvi pod mišični sloj in nad transverzalno fascijo v podganah

Histological advantages of two-component fibrin mesh (TachoSil) compared with polypropylene mesh after placement in the inguinal region in laboratory rats

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Izvleček

Operacije hernije so ena izmed najbolj pogostih operacij tako v veterinarski kot človeški medicini in na voljo je široka izbira glede mreže, ki jo kirurgi uporabljajo za popravo transverzalne fascije. Primerjali smo dvo-komponentno fibrinsko mrežo TachoSil in polipropilensko mrežo pri rekonstrukciji transverzalne fascije po vstavitvi pod mišični sloj in nad transverzalno fascijo pri podganah. Podganam vrste Fischer je bila vstavljena ali polipropilenska ali fibrinska mrežica pod mišični sloj in nad transverzalno fascijo. 30 dni po posegu so bile podgane žrtvovane, fibrinska ploščica z mrežicama je bila odstranjena ter poslana na histološko analizo. Ugotovili smo, da so podgane z dvo-komponentno mrežico imele manj pooperativnih komplikacij, ter manjšo prisotnost vnetnih celic, kar je povezano z večjo verjetnostjo za uspešno popravilo hernije.

Abstract

Hernia repair surgeries are among the most common procedures in both veterinary and human medi-cine, with various mesh options available for surgeons repairing the transversalis fascia. This study compared a variant of two-component fibrin mesh (TachoSil) and polypropylene mesh in reconstruct-ing the transversalis fascia by placing them in the inguinal region in Fischer laboratory rats. Specifically, the TachoSil or fibrin mesh was implanted into the groin region of Fischer rats, and after 30 days of mesh placement, the rats were sacrificed, the fibrous plate with either inserted mesh removed, and the tissue sent for histological analysis. The results showed that, compared with the polypropylene mesh, the implanted TachoSil mesh resulted in fewer postoperative complications and lower inflammatory cell presence.

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INTRODUCTION

The two-component fibrin net is widely used today for various indications and was first introduced in surgery in the early 1990s. Initially, this mesh consisted of equine collagen, bovine thrombin, aprotinin, and human fibrinogen. Over time, animal-derived components were replaced with human-derived components, and the latest generations, including the one we used in our study (TachoSil) no longer contain bovine components. To date, four randomized clinical studies have demonstrated the effectiveness of said two-component fibrin mesh (TachoSil) in liver, urological, and thoracic surgeries [1-6]. The physical and mechanical properties of this mesh are particularly noteworthy. Wet TachoSil mesh is approximately 2.5 times more elastic than the dry version, allowing it to adhere precisely to tissues and organs, move smoothly over surfaces, and withstand extreme stretching and stress [7]. However, polypropylene mesh remains the most commonly used prosthetic implant in abdominal hernia surgery due to its strength and integration into surrounding tissue. Although this integration is desirable because the mesh forms a solid fibrous plate that prevents recurrence, it can also cause severe inflammatory reactions and solid adhesions during scar formation [8]. New biomaterials are continually being developed to reduce the inflammatory response. Such materials have played a key role in the implantable device industry. New biomaterials with built-in anti-inflammatory properties have already shown significant success, reducing both inflammation and adhesive interactions [9]. It has been hypothesized that reducing the amount of polypropylene in the mesh can lower the inflammatory response during the postoperative period. The severity of the inflammatory response to the foreign body type cells and production of scar tissue depend on the structure of the incorporated material. Meshes with reduced polypropylene content, greater elasticity, and larger pores may offer significant advantages [10-12]. Despite the clear benefits of biomaterials, the use of prosthetic meshes in hernia surgery remains widespread. The primary reasons include the lack of definitive evidence, concerns about erosion into visceral organs, formation of adhesions, and chronic groin pain [13]. Meshes should prioritize the safety and long-term reliability of existing implants, especially with dominant scar formation and good tissue integration. However, this is not the case, as findings from animal models using "heavy" polypropylene meshes, have shown complications such as seromas, infections, mesh compression, and migration.

MATERIALS AND METHODS

This research was conducted using Fischer strain rats in the Laboratory for Medical Biology at the Faculty of Medicine, University of Zagreb, (Zagreb, Croatia) between 2006 and 2008.

A total of 78 rats (38 female, 49%; 40 male, 51%) were included, all 3 months old and weighing between 300 and 350 g. The animals were divided into two groups: the first group [N = 40; 23 males (57.5%) and 17 females (42.5%)] was treated with a polypropylene mesh measuring 2 × 1.5 cm^2 inserted into the groin region above the transversalis fascia, whereas the second group [N = 38; 29 males (76.3%) and 9 females (23.7%)] was treated with a 2 × 1.5 cm^2 two-component fibrin mesh (TachoSil sealant matrix, Corza Medical GmbH, Austria) inserted into the same region (Fig. 1).

Procedure

The rats were first shaved and their skin disinfected with alcohol, after which they were then anesthetized using ether and placed in the supine position. Next,

Figure 1. *Fisher rats in laboratory conditions.*

Figure 2. *Sacrificed rats after fibrotic plaque removal.*

a 3-cm skin and subcutaneous incision was made in the groin region, exposing the muscular layer through careful preparation with scissors. Depending on the group, either the polypropylene or TachoSil mesh was then placed below the muscle layer and above the transversalis fascia. The wounds were closed using Michel's clips (Fig 2.), after which the animals were allowed to recover under standard conditions and monitored for 30 days. The postoperative variables we observed included bleeding, hematoma, wound and implant infections, and mobility within the first 24 h and after 24 h. After 30 days, the animals were sacrificed and tissue specimens collected for histological analysis at the Department of Pathology and Cytology, Clinical Hospital Merkur Zagreb (Fig. 3). Histological analysis included measuring the presence of leukocytes and foreign-body cells using microscopy.

Statistical analysis

The data, including presence of leukocytes and foreign body cells, were analyzed using descriptive statistics, including arithmetic mean, standard deviation, median, and range. The chi-square test was applied to compare qualitative characteristics such as inflammation and the presence of foreign body type cells. For smaller samples, Fisher's exact test was used. As the data did not follow a Gaussian distribution, quantitative comparisons between the polypropylene and fibrin groups were conducted using the Mann–Whitney U test for two independent samples.

RESULTS

In the first group of animals (40 rats treated with polypropylene mesh), bleeding and hematoma in the wound were observed in six (15.0%) and four (10.0%) animals within the first 24 h and after 24 h, respectively. Wound and implant infections occurred in two (5.0%) animals. Mobility was recorded in 20 (50.0%) and 8 (20.0%) animals in the first 24 h and after 24 h, respectively. Importantly, in the second group of animals (38 rats treated with TachoSil mesh) bleeding and hematoma in the wound were not recorded at any time point nor were implant (mesh) infections observed. Finally, mobility in the first 24 h was observed in 32 (84.2%) animals, whereas mobility after 24 h was observed in 6 (15.8%) animals.

Figure 3. *Fibrotic plaque tissue after removal for histological analysis, containing either polypropylene or TachoSil mesh.*

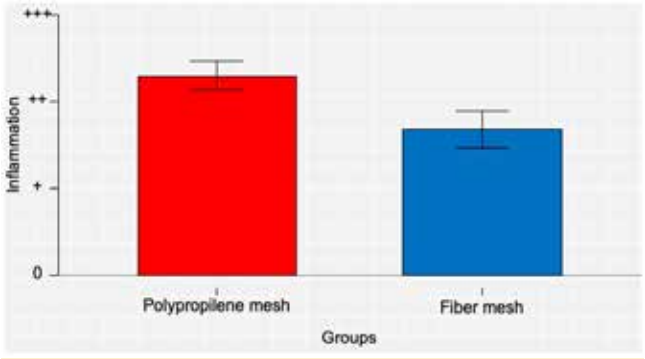

Figure 4. *Distribution of groups based on the presence of inflammation. +, Poor; ++, moderate; +++, strong.*

Table 1: Distribution of animals by presence of Inflammation

			Inflammation			Total
			+	++	+++	
Groups	Polypropylene Mesh	Number Of Individuals	1	26	13	40
		% Groups	2,5%	65,0%	32,5%	100,0%
		% Inflammation	5,9%	59,1%	76,5%	51,3%
	Fibrin Mesh	Number Of Individuals	16	18	4	38
		% Groups	42,1%	47,4%	10,5%	100,0%
		% Inflammation	94,1%	40,9%	23,5%	48,7%
Total		Number Of Individuals	17	44	17	78
		% Groups	21,8%	56,4%	21,8%	100,0%
		% Inflammation	100,0%	100,0%	100,0%	100,0%

Histological analyses

The results of histological analyses of the presence of inflammatory and foreign body type cells, are displayed graphically and in tables 1, 2 and 3. The results of the fibrous plate analysis indicated most rats treated with TachoSil mesh had moderate or less inflammation, as measured by the presence of leukocytes in the histological tissue. This finding highlighted the advantage of using the TachoSil mesh over the polypropylene mesh (Fig. 4 and Tables 1 and 3). Most rates with the polypropylene mesh also had a significantly higher presence of foreign body type cells than those with TachoSil mesh (Table 2).

DISCUSSION

This study compared variables related to complications and fibrous plaque formation following the implantation of either polypropylene or fibrin mesh in the inguinal region of rats. The results indicated a clear advantage of the two-component fibrin mesh compared with the polypropylene mesh. The statistical analysis revealed a significant difference in inflammation levels and presence of foreign body type cells, with the fibrin mesh group showing fewer postoperative complications in the two-component fibrin mesh rat group.

The polypropylene group exhibited abundant chronic inflammatory infiltrates, including lymphocytes, plasma cells, eosinophilic granulocytes, and mast cells. In contrast, the TachoSil mesh group showed only mild inflammatory infiltration (Fig. 4 and Tables 1 and 3). Additionally, the number of foreign body type cells were significantly higher in the polypropylene group compared with the TachoSil group (Table 2). This shows a significant favor in terms of postoperative healing in the TachoSil group, which in turn means a greater chance of a successful hernioplasty repair with a smaller chance of hernia recurrence.

The extensive chronic inflammatory response in the polypropylene group indicated poorer healing and less favorable implant outcomes. Conversely, the reduced presence of foreign body type cells and inflammation in the TachoSil group suggested that the two-component fibrin mesh was a more biologically compatible material compared with the polypropylene mesh which again leads to a greater chance of a successful repair.

Table 2. Distribution of animals based on the presence of foreign body type cells

			Foreign Body Type Cells			Total
			+	++	+++	
Groups	Polypropylene Mesh	Number Of Individuals	1	24	15	40
		% Groups	2,5%	60,0%	37,5%	100,0%
		% Foreign Body Type Cells	4,8%	57,1%	100,0%	51,3%
	Fibrin Mesh	Number Of Individuals	20	18	0	38
		% Groups	52,6%	47,4%	0%	100,0
		% Foreign Body Type Cells	95,2%	42,9%	0%	48,7%
Total		Number Of Individuals	21	42	15	78
		% Groups	26,9%	53,8%	19,2%	100,0%
		% Foreign Body Type Cells	100,0%	100,0%	100,0%	100,0%

Table 3: Presence of inflammation in the stady gruops

		Groups		Total
		Polypropylene Mesh	Fibrin Mesh	
Inflammation	+	1	16	17
	++	26	18	44
	+++	13	4	17
Total		40	38	78

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Kakovost življenja slovenskih otrok in mladih odraslih z redkimi boleznimi ledvic

Health-related Quality of Life in Slovenian Pediatric and Young Adult Patients with Rare Kidney Diseases

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Izvleček

Namen: *Pacienti z redkimi boleznimi ledvic potrebujejo posebno zdravstveno obravnavo in neredko doživljenjsko zdravljenje. Pomemben aspekt je njihova kakovost življenja, na katero vplivajo številni dejavniki. V tej presečni študiji smo nameravali oceniti kakovost življenja otrok in mladih odraslih z redkimi ledvičnimi boleznimi, da bi lahko izboljšali njihovo oskrbo. Članek izhaja iz naše raziskovalne naloge »Kvaliteta življenja otrok z redkimi boleznimi ledvic in njihovo zdravljenje«, ki je 6. 12. 2024 prejela Dekanovo priznanje za študente.*

Metode: *Študija je potekala pod vodstvom Klinike za pediatrijo Univerzitetnega kliničnega centra (UKC) Maribor in je vključila 2–25-letne bolnike z redkimi*

Abstract

Purpose: *Patients with rare kidney diseases require specialized and typically lifelong treatments. Quality of life is an important aspect of a person's well-being, and it is influenced by numerous factors. This cross-sectional study aimed to assess the quality of life of affected children and young adults to guide optimal treatment and enhance care. It is based on our research paper that was awarded the Dean's Recognition for Students on December 6, 2024.*

Methods: *The study included patients aged 2–25 years with rare kidney diseases who were managed in the Department of Pediatrics at the University Clinical Centre Maribor and registered in the European Rare Kidney Disease Registry. The Pediatric Quality of Life Inven-*

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boleznimi ledvic iz registra ERKReg (angl. European Rare Kidney Disease Registry). Udeleženci oziroma njihovi starši so izpolnili standardiziran vprašalnik o kvaliteti življenja otrok (angl. PedsQL). Analizirali smo 69 vprašalnikov otrok in 58 vprašalnikov staršev. Rezultati so predstavljeni z deskriptivnimi metodami. Za določitev razlik v funkcioniranju med starostnimi skupinami smo uporabili Kruskal-Wallisov test in Dunnov post-hoc test z Bonferronijevo korekcijo. Odgovore staršev in otrok smo primerjali s pomočjo t-testa za parne vzorce.

Rezultati: *Otroci so skupno kakovost življenja ocenili z mediano 82,5 (IQR = 17,45), njihovi starši pa s 83,91 (IQR = 17,85), tj. od skupno 100 možnih točk. Mladostniki bolje funkcionirajo na socialnem področju kot mlajši otroci (H = 11,335; p = 0,023). Odgovori staršev se ujemajo z odgovori otrok na fizičnem (t = -0,354; p = 0,725), čustvenem (t = -0,574; p = 0,569), socialnem (t = 0,061; p = 0,951), šolskem (t = -0,271; p = 0,787) in skupnem področju (t = -0,765; p = 0,448).*

Zaključek: *Udeleženci imajo dobro splošno kakovost življenja, kar poudarja pomen dosedanje in prihodnje multidisciplinarne oskrbe ter nadaljnjih intervencij za preprečevanje morebitnega poslabšanja.*

tory questionnaire was sent to the participants and their parents as proxy reports, and 69 patient questionnaires and 58 parent questionnaires were obtained for analysis. Descriptive methods were used to present the results. The Kruskal–Wallis and Dunn's post hoc test with Bonferroni correction were used to determine functioning differences among different age groups, whereas the paired-samples t test was used to compare parent–child responses.

Results: *Patients rated their overall quality of life with a median of 82.5 [interquartile range (IQR) = 17.45], whereas parents rated it with a median of 83.91 (IQR = 17.85), out of 100. Adolescents had better social functioning than young children (H = 11.335; P = 0.023). Parents' reports aligned with those of children [physical (t = −0.354; P = 0.725), emotional (t = −0.574; P = 0.569), social (t = 0.061; p = 0.951), school (t = −0.271; P = 0.787), and total functioning (t = −0.765; P = 0.448)].*

Conclusion: *Participants had good overall functioning, highlighting the importance of multidisciplinary care and the need for further interventions to enhance health and prevent potential deterioration.*

INTRODUCTION

Rare kidney diseases are a heterogeneous group of at least 150 medical conditions characterized by a chronic, progressive, and often degenerative course. Patients with these diseases require specialized medical care with lengthy diagnostic procedures and, in many cases, lifelong treatment (1, 2). These conditions significantly impact the quality of life of patients, making good treatment adherence crucial for achieving optimal health outcomes (3, 4).

Rare kidney diseases negatively influence the physical functioning of children (5, 6). Pediatric patients with these conditions are less physically active than their healthy peers, resulting in poorer physical performance (5, 6). They also lack confidence, skills, and motivation to participate in physical activities (5). Although the connection between these diseases and emotional functioning is well-known, emotional aspects often receive inadequate attention (7). Children with rare kidney diseases are more likely to develop mental health issues, including anxiety, depression, and adjustment disorders, compared with their healthy peers (3). Hence, the focus should be on prevention, early detection, and provision of sufficient psychological support (8–10).

Rare kidney diseases also impair social functioning (10, 11). Affected children often experience limitations in their ability to socialize with peers, engage in sports, and participate in school trips due to frequent medical visits and treatments (10, 11). This leads to smaller social networks and increased isolation, making peer acceptance an even greater challenge for these children (10). Moreover, managing disease

complications (e.g., urinary incontinence) is essential because they can be disruptive, cause discomfort, lower self-esteem, and increase social isolation (10, 12). Finally, these diseases negatively influence neurological development (13). Approximately 20%–25% of children aged less than 5 years with grade 5 chronic kidney disease experience general developmental delays, including difficulties with attention, executive functions, and visual–spatial abilities (13). Previous studies indicated that kidney transplant recipients scored lower in math and reading compared with their healthy peers but performed better than those on dialysis (13, 14).

Health-related quality of life in pediatric patients with rare kidney diseases remains less explored in Slovenia, which motivated us to conduct this study to evaluate the physical, emotional, social, and academic functioning of these patients, along with parental perceptions. We assessed the following hypotheses:

1. The overall quality of life score is less than 75, according to the PedsQL questionnaire.
2. Parents rate their children's quality of life lower than the children themselves.

We hope this study will improve care strategies and enhance the quality of life of pediatric patients with rare kidney diseases.

MATERIAL AND METHODS

Study design, setting, and patient sample

The cross-sectional study was coordinated by the Department of Pediatrics at University Medical Centre (UKC) Maribor, Slovenia, and received ethical approval in November 2023 (15). In December 2023, we acquired a list of 152 patients with rare kidney diseases also included in the European Rare Kidney Disease Registry. All patients met the inclusion criteria: (a) age between 2 and 25 years; (b) diagnosis of rare kidney disease; and (c) mental ability to complete the questionnaire. We contacted the parents or patients directly, explained the background and purpose of the study, and obtained their oral consent. Instructions were also provided. A total of 114 parents responded to the call. Standardized PedsQL 4.0 questionnaires were sent to participating children, young adults, or their parents (16). Each parent received two questionnaires (except for those of toddlers and young adults, who received one). One was for the parents, and the other was for their child. The data were collected from January 2024 to June 2024 (15).

Measures

We used the PedsQL Generic Core Scales version 4.0 in Slovenian translation. It comprised parallel patient self-report and parent proxy-report questionnaires: age 5–7 years for young children, 8–12 years for children, and 13–18 years for adolescents (17). We also used the parent proxy report for toddlers (age 2–4 years) and the self-report for young adults (age 18–25 years). All mentioned questionnaires are freely available for nonfunded academic research, which include those used in our study, on the ePROVIDE Mapi Research Trust website (16). The questionnaires comprise 23 items assessing physical (8 items), emotional (5 items), social (5 items), and school (5 items) functioning of respondents, focusing on the frequency of specific problems in the last month (17). Respondents used a 5-point response scale, whereas younger children used a 3-point scale. Surveys with more than 50% missing responses were considered invalid (18). We used all received questionnaires (69 patient self-reports and 58 parent proxy reports) for analysis (15). The survey items were linearly converted into a 0–100 scale: 0 = 100, 1 = 75, 2 = 50, 3 = 25, and 4 = 0 (5-point scale) or 0 = 100, 2 = 50, and 3 = 0 (3-point scale), where higher scores indicated better functioning (18). Individual categories of scale scores were combined into a total scale score (18).

Outcomes

Primary outcomes included physical functioning, emotional functioning, social functioning, school functioning, and total scale score. Secondary outcomes included parents' responses. Sex, illness duration, and comorbidities were not considered.

Statistical analysis

We used JASP (Jeffreys's Amazing Statistics Program), version 0.18.3, to calculate descriptive statistics and

the Shapiro–Wilk test to assess the normality of distribution. Nominal variables were presented as numbers and percentages (%), and numerical variables as the median and interquartile range (IQR). We used the Kruskal–Wallis test and Dunn's post hoc test with Bonferroni correction to determine differences in quality of life among patients with different age groups. We compared parent–child responses using the paired-samples t test.

Ethical statement

The study was approved by the Committee for Medical Ethics at the University Clinical Centre Maribor (No. UKC-MB-KME-54/23, 30 November 2023). We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed in the study.

RESULTS

Sample characteristics

A total of 114 parents consented to participate in our study. Of the 202 questionnaires sent, we received 69 patient self-reports (60.5% response rate) and 58 parent proxy reports (65.9% response rate), all were considered valid for the study. Of the 69 patients, 8 were toddlers (11.6%), 6 young children (8.7%), 21 children (30.4%), 22 adolescents (31.9%), and 12 young adults (17.4%). Of the 58 parents, 6 had young children (10.3%), 25 had children (43.1%), and 27 had adolescents (46.6%).

The distribution of rare kidney disease groups among participants, classified according to the list published on the ERKNet website, is presented in Table 1 (19).

Quality of life across all patients and age groups

The Shapiro–Wilk test indicated an abnormal distribution of individual and total categories of scale scores in the patient group [physical ($W = 0.805$; $P < 0.001$), emotional ($W = 0.944$; $P = 0.004$), social ($W = 0.811$; $P < 0.001$), school ($W = 0.928$; $P < 0.001$), and total functioning ($W = 0.929$; $P < 0.001$)] and the parent group [physical ($W = 0.722$; $P < 0.001$), emotional ($W = 0.902$; $P < 0.001$), social ($W = 0.806$; $P < 0.001$), school ($W = 0.920$; $P = 0.003$), and total functioning ($W = 0.900$; $P < 0.001$)].

The scale score categories and total scale score for all patients and by age group, expressed as the median (IQR), including both patient and parent responses, are shown in Table 2, along with the paired-samples t test results.

Differences in quality of life among different age groups

The Kruskal–Walli's test results showed no significant difference in the quality of physical ($H = 2.413$; $P = 0.660$), emotional ($H = 6.410$; $P = 0.171$), school ($H = 3.413$; $P = 0.491$), and total functioning ($H = 4.453$; $P = 0.348$) based on the age group. However, social functioning did differ based on the age

Table 1. Distribution of rare kidney disease groups among participants

Disease group	Age group				
	Toddlers (n = 8)	Young children (n = 6)	Children (n = 21)	Adolescents (n = 22)	Young adults (n = 12)
Glomerulopathies	/	*n* = 3	*n* = 3	*n* = 6	*n* = 5
Tubulopathies	/	/	*n* = 1	*n* = 1	*n* = 1
Renal or urinary tract malformations	*n* = 7	*n* = 2	*n* = 14	*n* = 9	*n* = 3
Familial cystic renal diseases	*n* = 1	*n* = 1	*n* = 2	*n* = 5	*n* = 3
Thrombotic microangiopathies	/	/	/	*n* = 1	/
Rare causes of hypertension	/	/	*n* = 1	/	/

n, Number of participants.

Table 2. Presentation of the quality of life with median (IQR)

	Patient Self-Report	Parent Proxy-Report	Paired-Sample-T-test			
			t	p	MD	95 CI
5-7 years						
Physical functioning	90.63 (15.63)	98.44 (13.84)	-0.213	0.839	-4.17	-54.4; 46
Emotional functioning	75 (17.5)	72.5 (26.25)	0.117	0.912	1.67	-35.1; 38.4
Social functioning	75 (17.5)	87.5 (28.75)	-1.954	0.108	-16.67	-38.6; 5.3
School functioning	75 (25)	80 (15)	-0.201	0.849	-2.5	-34.5; 29.5
Total functioning	78.59 (6.8)	83.36 (18.71)	-0.589 -	0.582	3.91	-21; 13.2
8-12 years						
Physical functioning	93.75 (15.63)	93.75 (12.5)	-0.207	0.638	-0.89	-9.9; 8.1
Emotional functioning	80 (25)	80 (15)	-0.478	0.638	-2.86	-15.3; 9.6
Social functioning	90 (25)	85 (25)	0.312	0.758	1.73	-9.8; 13.3
School functioning	80 (15)	80 (15)	0.890	0.384	2.86	-3.8; 9.6
Total functioning	86.25 (17.81)	83.44 (12.81)	-0.322	0.751	-0.71	-5.3; 3.9
13-18 years						
Physical functioning	90.63 (17.9)	93.75 (10.94)	-0.176	0.862	-0.95	-12.1; 10.2
Emotional functioning	77.5 (30)	85 (35)	0.472	0.642	-2.96	-16; 10.1
Social functioning	100 (15)	100 (25)	0.582	0.567	3.41	-8.8; 15.6
School functioning	76.25 (42.5)	80 (35)	-0.631	0.535	-4.21	-18.1; 9.6
Total functioning	83.67 (17.75)	83.9 (24.63)	0.423	0.677	-1.1	-6.5; 4.3
Entire sample						
Physical functioning	93.75 (21.87)	93.75 (12.5)	-0.354	0.725	-1.32	-8.8; 6.2
Emotional functioning	70 (30)	80 (28.75)	-0.574	0.951	-2.35	-10.6; 5.9
Social functioning	90 (25)	95 (25)	0.061	0.951	0.23	-7.3; 7.7
School functioning	75 (26.67)	80 (23.75)	-0.271	0.787	-0.97	-8.2; 6.2
Total functioning	82.5 (17.45)	83.91 (17.85)	-0.765	0.448	-1.28	-4.6; 2.1

CI, Confidence interval; MD, mean difference; t, value of the statistical test.

group (H = 11.335; P = 0.023). Dunn's post hoc test with Bonferroni correction revealed better social functioning among adolescents than young children (P = 0.023).

Differences between patient self-report and parent proxy-report

The Shapiro–Wilk test [(physical (W = 0.958; P = 0.079), emotional (W = 0.986; P = 0.812), social (W = 0.984; P = 0.729), school (W = 0.970; P = 0.249), and total functioning (W = 0.984; P = 0.742)) indicated the normal distribution of the differences in responses between patient self-reports and parent proxy-reports. Therefore, the parametric version of the paired-samples t test was used (Table 2). The responses of parents and their children did not differ significantly in any of the examined fields (P > 0.05).

DISCUSSION

Quality of life of the entire sample of patients

Our results suggested that Slovenian children and

young adults with rare kidney diseases reported a good overall quality of life. Patients rated it with a median of 82.5 (IQR = 17.45), whereas their parents rated it at 83.91 (IQR = 17.85). Consequently, we could reject the first hypothesis, which predicted that the overall quality of life score would be less than 75. Our findings were comparable to those of previous studies (20, 21). Minor discrepancies might be attributed to the varying characteristics of the included participants and the varying standards of living across different countries. The positive overall functioning could be attributed to medical advances, including comprehensive healthcare, support in school and social areas, psychosocial support, and other adjustments that help children manage daily challenges (12).

Participants rated physical functioning the highest and emotional functioning the lowest, whereas parents rated social function the highest and emotional the lowest. This was consistent with previous findings, as poor emotional functioning was influenced by numerous factors (3). Differences in how children and their parents perceived the difficulties highlighted various aspects of living with the disease (22). Children might perceive physical functioning as better because they developed coping strategies and accepted disease limitations as normal (22). Additionally, we believe that physical difficulties are more apparent than social ones. This is because children often do not communicate their social struggles, which makes parents underestimate their severity.

Quality of life across different age groups

Young children exhibit poorer social functioning than adolescents, which can be explained in many ways (23). On the one hand, bullying is mentioned as being more prevalent in childhood than in adolescence (24). On the other hand, adolescents are more independent than young children, who are either not yet in or just beginning the phase of secondary socialization (23). The presence of a chronic illness can hinder this process because various impairments negatively impact self-esteem, and, consequently, relationships with peers (3). Moreover, the brain undergoes remodeling during adolescence; hence, neural plasticity may facilitate the development of social cognitive skills (25).

Comparison of patient self-report and parent proxy-report

Information provided by proxies, most often parents, does not always align with the information reported by the patients themselves (26). Discrepancies, to some extent, are not surprising because a child's perception of quality of life often differs from that of their parents (26). Eiser and Morse found that the greatest differences in responses appeared in the areas of emotional and social functioning, whereas the highest agreement was observed in physical functioning (27). However, other researchers believe that this is not always the case (28, 29). Therefore, our results are noteworthy because we did not find significant differences between the responses, contradicting our second hypothesis. Harmer et al. also discovered a similarly strong correlation, possibly due to the relatively small sample size (30).

Parents of young children believed that their children experienced the fewest difficulties with emotional functioning, whereas the children perceived school functioning as their greatest challenge. This nonsignificant discrepancy might be due to the children's limited awareness of the complexity of their emotional problems (25).

The responses of children aged 8–12 years and adolescents aligned with those of their parents. This high level of agreement between adolescents and parents contradicted previous findings, including the one by Nap-van der Vlist et al. (31).

Limitations and future research

A significant limitation of our study was the variability of rare kidney diseases among participants, which differed in clinical presentation, severity, and prognosis. Besides chronic illness, numerous other factors, not considered in our study, affect the quality of life of patients. Therefore, future research should account for multifactorial influences. The questionnaires were sent to the participants, making it challenging to strictly adhere to the instructions. It is possible that parents completed or at least influenced the responses of their children. We suggest administering the questionnaire during a follow-up visit for future studies, although it may require additional time and effort from healthcare workers.

CONCLUSIONS

The participants rated their quality of life at 82.5 out of 100 on the PedsQL questionnaire, indicating good overall functioning. The study reported no significant differences in physical, emotional, school, and overall functioning across different age groups. However, social functioning was better among adolescents compared with young children. Additionally, parents' reports aligned with those of their children. The study also highlighted the need for increased therapeutic engagement in emotional support and treatment. Finally, it reaffirmed the value of multidisciplinary care, treatment, and monitoring of these patients.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Validacija slovenske različice vprašalnika Patient-Practitioner Orientation Scale – PPOS

Validation of the Slovenian Version of the Patient-Practitioner Orientation Scale – PPOS

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Izvleček

Namen: *Koncept zdravstvene oskrbe, ki pacienta postavlja v središče, se je razvil v zadnjih desetletjih. Nov pristop, imenovan oskrba, usmerjen na pacienta, poudarja pomen razumevanja in upoštevanja individualnih potreb, vrednot in želja pacienta ter spodbuja aktivno sodelovanje med pacientom in zdravstvenimi delavci na vseh ravneh. Ta pristop vključuje skrb za psihološke, socialne in duhovne potrebe pacientov. Za merjenje oskrbe, usmerjene na pacienta, je bilo razvitih več vprašalnikov, med katerimi je pogosto uporabljen Krupatov vprašalnik Patient-Practitioner Orientation Scale (PPOS).*
Metode: *Raziskava validacije vprašalnika PPOS je bila izvedena v več fazah. Prevod PPOS lestvice je potekal v skladu s priporočili Svetovne zdravstvene organizacije in smernicami International Society for Pharmacoeconomics and*

Abstract

Purpose: *Recent decades have seen the emergence of a healthcare framework that places the patient at the core, giving rise to a novel approach termed patient-centered care (PCC) which emphasizes the importance of understanding and respecting the individual needs, values, and preferences of patients, while promoting active collaboration between patients and healthcare providers at all levels. This approach also addresses the psychological, social, and spiritual needs of patients. Several questionnaires have been developed to measure PCC, among which Krupat's *Patient-Practitioner Orientation Scale* (PPOS) is frequently used.*
Methods: *Validation of the PPOS questionnaire was conducted in several phases. The translation followed the recommendations of the World Health Organization and the ISPOR guidelines. The process in-*

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Outcomes Research (ISPOR). Postopek je vključeval naslednje: prevod vprašalnika v slovenski jezik s strani dveh neodvisnih raziskovalcev, pregled in uskladitev prevodov s strani strokovnjakov, povratni prevod vprašalnika v angleščino in kulturološko preverjanje ustreznosti vprašalnika. Vprašalnik je bil poslan na e-naslove zdravnikov družinske medicine, ki so sodelovali v raziskavi. Sledila je eksploratorna faktorska analiza podatkov in preverjanje notranje skladnosti lestvice.

Rezultati: *Vprašalnik je izpolnilo 112 zdravnikov družinske medicine različnih starosti, delovnih okolij in velikosti ambulant. Kriteriju „v pacienta usmerjen" sta zadostili v vprašalniku samo postavki 9 in 13. „Zmerna usmerjenost v pacienta" se je pokazala v postavki 17. Vse ostale postavke so izpolnile kriterij „usmerjenosti v zdravnika". Z eksploratorno faktorsko analizo so bili ugotovljeni štirje faktorji lestvice – za vsakega od teh je bila preverjena zanesljivost. Izračun Cronbach alpha je prikazal sprejemljivo notranjo skladnost za celotni vprašalnik (0,724), prav tako za faktor 1 (0,709) in faktor 2 (0,688), pri drugih dveh faktorjih pa nizko notranjo skladnost (faktor 3 = 0,437, faktor 4 = 0,437). Pri dveh je izračun Cronbach alpha prikazal sprejemljivo notranjo skladnost (faktor 1 = 0,709, faktor 2 = 0,688), pri drugih dveh pa nizko (faktor 3 = 0,437, faktor 4 = 0,437).*

Zaključek: *Analiza rezultatov je pokazala pretežno usmerjenost v zdravnika. Validacijski postopek je pokazal, da slovenski prevod vprašalnika v trenutni obliki za uporabo ni primeren. Potrebne so nadaljnje prilagoditve in dodatna validacija, da bi bil vprašalnik ustrezno orodje za oceno oskrbe, usmerjene na pacienta, v slovenskem okolju.*

cluded: translation of the questionnaire into Slovenian by two independent translators, review and harmonization of the translations by experts in the field of family medicine, back-translation of the questionnaire into English, and cultural validation of the questionnaire's appropriateness. The questionnaire was distributed via email to participating family medicine physicians. This was followed by exploratory factor analysis of the data and an assessment of the scale's internal consistency.

Results: *The questionnaire was completed by 112 family physicians of various ages, working in different environments and practice sizes. Only items 9 and 13 in the questionnaire met the criterion of being "patient-oriented." "Moderate patient orientation" was observed in item 17. All other items met the criterion of being "practitioner-oriented." Exploratory factor analysis identified four factors within the scale. The reliability of each of the four factors was assessed. The calculation of Cronbach's alpha showed acceptable internal consistency for the entire questionnaire (0.724), as well as for factor 1 (0.709) and factor 2 (0.688). For the other two factors, however, low internal consistency was observed (factor 3 = 0.437, factor 4 = 0.437).*

Conclusion: *The analysis of the results revealed a predominant orientation toward the practitioner. The validation process showed that the Slovenian translation of the questionnaire in its current form is not yet suitable for use. Further adjustments and additional validation are needed to ensure that the questionnaire becomes an appropriate tool for assessing PCC in the Slovenian context.*

INTRODUCTION

In recent years, healthcare has undergone a significant transformation, shifting from a disease-centered model to one that places the patient at the core of care. This patient-centered approach is particularly emphasized in family medicine, where care extends beyond physical ailments to include the psychological, social, cultural, and existential dimensions of a person (1,2).

Unlike the traditional biomedical model that focused solely on diagnosing and treating diseases, patient-centered care (PCC) actively involves patients in clinical decision-making. It respects their values,

needs, and preferences, thereby fostering stronger patient-provider partnerships and more personalized, effective care (3,4). This holistic approach recognizes that health outcomes are influenced not just by medical interventions, but also by emotional well-being, social support, and environmental conditions (5,6).

One of the core elements of PCC is effective communication (7). Studies show that when patients feel heard and respected, they are more likely to follow treatment plans and achieve better outcomes (8). Additionally, including family members in care decisions improves overall satisfaction and helps address complex care needs, especially for chronic conditions (9). Research confirms that support from informal caregivers contributes to better health and smoother communication between healthcare providers and patients (10).

Successfully implementing PCC requires structural changes within healthcare systems. Institutions must foster a culture of respect, empathy, and collaboration, while also empowering healthcare workers through continuous education. Training programs focused on communication and empathy have been shown to support the adoption of PCC values (11-13).

Technology also plays a key role in enabling PCC. Tools like electronic health records improve information sharing and care coordination, although they bring challenges related to privacy and data security (14,15). Moreover, equitable access to care remains a concern, as socioeconomic and cultural factors can limit some patients' ability to fully participate in their healthcare (16).

Despite these challenges, the benefits of PCC are clear. It leads to better health outcomes, lower hospital readmissions, and higher satisfaction among patients and providers alike (17). Research supports the effectiveness of PCC, particularly in managing chronic and complex conditions (18).

To assess how well PCC is being implemented, various standardized questionnaires have been developed:

- The Patient-Practitioner Orientation Scale (PPOS), focusing on "Caring" and "Sharing" dimensions (11,19).
- The Person-Centered Care Assessment Tool (P-CAT) evaluates patient experiences, including communication and involvement in decision-making (20).
- The Person-Centered Coordinated Care Experience Questionnaire (P3CEQ) assesses care coordination and personalization across healthcare systems (21).
- The Generic Person-Centered Care Questionnaire (GPCCQ) is used in diverse healthcare settings to assess patient perception of PCC (22).

PCC represents a progressive and compassionate direction for modern healthcare. It requires system-level commitment, provider training, and validated tools to measure progress. Despite current challenges, this model offers promising improvements in health outcomes, patient satisfaction, and overall care quality (23).

In Slovenia, these tools are not yet widely validated. The validation of instruments like PPOS would allow Slovenian healthcare providers to assess and improve PCC practices effectively. Moreover, participating in international studies requires validated local versions of such tools, making their adaptation a priority.

The aim of this study was validation of the PPOS scale as a tool for use in the Slovenian healthcare system.

MATERIAL AND METHODS

Type of study and settings

The presented descriptive, cross-sectional, self-reported online study was part of a large international project titled "European General Practitioners'/Family Physicians' Attitudes Towards Person-Centered Care and Factors That Influence Its Implementation in Everyday Practice"* (PACE GP/FP study), across 24 European countries, among them Slovenia. The study was conducted in close collaboration with the European Association for Quality and Patient Safety in Primary Care (EQuiP) and the European General Practice Research Network (EGPRN) (24).

Participants

The study involved family physicians working in family medicine practices across Slovenia. The international PACE GP/FP study protocol set a minimum requirement of 100 participants per country to ensure sufficient statistical power of the

study (24). An invitation to participate was sent via the Medical Chamber of Slovenia to all email addresses of licensed family physicians and family medicine residents, totaling 1,178 addresses. The invitation was resent to the same recipients after 1 month. All family physicians who met the following inclusion criteria were eligible to participate: active work in a family medicine practice and willingness to participate and complete the questionnaire. Exclusion criteria included: absence due to illness or other reasons during the data collection period and failure to provide consent to participate in the study. Participating physicians self-identified whether their clinic operated in an urban or rural setting. A teaching clinic was defined as a clinic where the educational process for students, trainees, and residents takes place.

Data Collection

Data collection for this part of the study was conducted between March 2023 and January 2024 using the structured PPOS questionnaire, originally developed by Krupat et al. in 2000 (19, 25). The PPOS questionnaire is a doctor–patient orientation scale capable of assessing doctors', medical students' and patients' attitudes toward the doctor–patient relationship. The scale contains 18 items that reflect two domains related to the patient: Sharing and Caring. which are central to understanding physician-patient interactions. The nine-item Sharing domain assesses whether respondents believe that power and control should be shared between doctors and patients as well as the degree to which the doctor should share PPC. The Caring domain (also nine items) assesses the extent to which the doctor demonstrates warmth, support, and a patient-centered approach, reflecting attitudes towards empathy, understanding, and responsiveness to patients' feelings and needs.

Together, the Sharing and Caring domains provide a comprehensive measure of the orientation towards the doctor–patient relationship, capturing the balance between a more paternalistic, doctor-led approach and a more collaborative, patient-centered model.

Scores on the PPOS can range from a more practitioner-centered orientation (lower scores) to a more patient-centered orientation (higher scores). This scale has been widely used in cross-cultural studies to assess attitudes across different healthcare settings and populations (11).

We selected Krupat et al.'s PPOS scale due to its widespread use, robust psychometric properties, and its ability to capture physician attitudes along a continuum from practitioner-centered to patient-centered orientations.

Importantly, the PPOS was employed in the large-scale international PACE GP/FP study, providing a validated framework for cross-cultural comparison (24). Using the PPOS allowed us to align our study with this international project, facilitating comparability of results and contributing to a broader understanding of PCC attitudes across different healthcare systems (24).

The PPOS questionnaire was translated and adopted for use in a Slovenian context. The translation process followed the guidelines of the World Health Organization (WHO) and the International Society for Pharmacoeconomics and Outcomes Research (ISPOR) (26), and included:

- Translation of the questionnaire into Slovenian by two independent researchers.
- Harmonization of translations by experts in the field of family medicine.
- Back-translation into English to verify meaning.
- Cultural validation of the questionnaire's appropriateness.

Data collection took place via an online platform, where physicians received a link to complete the questionnaire. The collected data included:

- Independent variables: gender, age, work environment (rural/urban), and whether the clinic was a teaching institution.
- Dependent variables: PPOS questionnaire items measuring healthcare providers' orientation toward PCC.

Data Analysis

Data collected using the PPOS were analyzed using the statistical software IBM SPSS Statistics 29. The analysis methods included:

Descriptive statistics

Descriptive statistics were used to provide a basic overview of the sample, including the proportions of participants by gender, work environment (rural or urban), and the status of the clinic as a teaching institution. Frequencies, mean values, and standard deviations were calculated.

Descriptive analysis of the PPOS Questionnaire:

The questionnaire is scored on a scale from 1 to 6 points:

- 6 points: Strongly disagree
- 5 points: Moderately disagree
- 4 points: Slightly disagree
- 3 points: Slightly agree
- 2 points: Moderately agree
- 1 point: Strongly agree

Interpretation of the Questionnaire:

- Patient-oriented (>5.00)
- Moderately patient-oriented (4.57 – 5.00)
- Practitioner-oriented (<4.57)

Exploratory Factor Analysis:

To explore the underlying structure of the Slovenian version of the PPOS questionnaire, we conducted an exploratory factor analysis (EFA) using Principal Component Analysis (PCA) with varimax rotation. Indicators such as the number of factors, total explained variance, and factor loadings of the items were determined. PCA was chosen as an initial exploratory technique due to the limited sample size (n = 112), which constrained the use of more complex factor analysis methods such as common factor analysis. While we acknowledge that PCA does not distinguish between shared and unique variance and is more commonly used for data reduction rather than identifying latent constructs, it served as a preliminary step to identify potential factor groupings within the translated scale. The number of components retained was based on eigenvalues greater than 1 and examination of the scree plot.

Reliability Analysis:

Cronbach's alpha was calculated to assess the internal consistency of individual factors. Factors with alpha values above 0.7 were considered acceptably reliable, while factors with lower values indicated the need for adjustment of the sample.

- Values above 5 indicate a strong orientation towards PCC. Columns with mean values above 5 reflect a greater focus on the patient's needs and the adaptation of healthcare to the individual wishes of the patient.
- Values between 4.57 and 5 indicate a moderate orientation towards PCC. In these cases, physicians maintain a balanced approach between considering the patient's needs and their own professional judgment.
- Values below 4.57 suggest a more practitioner-oriented approach. This means that decisions during consultations are more influenced by the physician's professional judgment, with less consideration given to the patient's wishes.

RESULTS

A total of 112 family physicians who fully completed the PPOS questionnaire were included in the analysis. The response rate was 9.5%. The study involved more female than male physicians, with the majority working in urban settings and participating in the teaching process as mentors for students, trainees, and residents. The demographic characteristics of the participants are presented in Table 1.

Table 1. Demographic Characteristics of Family Physicians

Characteristics	NO	%
Male	34	30.4%
Female	78	69.6%
Rural settings	22	19.6%
Urban settings	90	80.4%
Teaching settings	87	77.7%
Non-teaching settings	25	22.3%

Descriptive Analysis of the PPOS Questionnaire

Table 2 shows the mean values of responses for each individual item or statement. The highlighted questions are those that indicated an orientation or partial orientation toward the patient.

Only Items 9 and 13 are in the patient-oriented range (>5.0):

- Item 9 (Patients should be treated as if they were partners...): 5.3
- Item 13 (Treatment plan must align with lifestyle/values): 5.2
- Item 17 (Humor is important) falls in the moderately patient-oriented range: 4.6

The remaining 15 items are all practitioner oriented.

Validity of the PPOS Questionnaire

In the analysis of the PPOS questionnaire, we used a four-factor model, where the individual factors explained a total variance of 47.6%.

Exploratory Factor Analysis of the PPOS Scale

The 18 items of the questionnaire were distributed across four factors. The distribution of items by factors is shown in Table 3. The contributions of individual statements are presented in Table 4.

Table 2: Descriptive analysis of PPOS

No.	Item	Min.	Max.	Mean	SD*
1	The doctor is the one who should decide what gets talked about during a visit.	1	6	3.2	1.5
2	Although healthcare is less personal these days, this is a small price to pay for medical advances.	1	6	2.7	1.4
3	The most important part of the standard medical visit is the physical exam.	1	6	3.1	1.5
4	It is often best for patients if they do not have a full explanation of their medical condition.	1	6	2.1	1.2
5	Patients should rely on their doctors' knowledge and not try to find out about their conditions on their own.	1	6	3.1	1.5
6	When doctors ask a lot of questions about a patient's background, they are prying too much into personal matters.	1	6	1.9	1.1
7	If doctors are truly good at diagnosis and treatment, the way they relate to patients is not that important.	1	6	1.6	1.1
8	Many patients continue asking questions even though they are not learning anything new.	1	6	3.7	1.3
9	Patients should be treated as if they were partners with the doctor, equal in power and status.	4	6	5.3	1.5
10	Patients generally want reassurance rather than information about their health.	1	6	3.8	1.2
11	If a doctor's primary tools are being open and warm, the doctor will not have a lot of success.	1	6	2.2	1.3
12	When patients disagree with their doctor, this is a sign that the doctor does not have the patient's respect and trust.	1	6	2.8	1.3
13	A treatment plan cannot succeed if it is in conflict with a patient's lifestyle or values.	4	6	5.2	1.2
14	Most patients want to get in and out of the doctor's office as quickly as possible.	1	6	3.8	1.7
15	The patient must always be aware that the doctor is in charge	1	6	2.7	1.5
16	It is not that important to know a patient's culture and background in order to treat the person's illness.	1	5	1.9	1.1
17	Humour is a major ingredient in the doctor's treatment of the patient.	4	6	4.6	1.2
18	When patients look up medical information on their own, this usually confuses more than it helps.	1	6	4.4	1.2

SD – standard deviation*

Table 3. Exploratory Factor Analysis of the PPOS Scale

Item	Factor 1	Factor 2	Factor 3	Factor 4
1	0.674	0.198	-0.096	-0.118
2	0.650	0.221	-0.144	0.181
3	0.686	0.150	0.010	0.001
4	0.210	0.478	-0.332	0.021
5	0.242	0.725	-0.012	-0.022
6	0.519	-0.233	-0.205	0.459
7	0.489	-0.079	-0.262	0.394
8	0.563	0.185	0.240	0.002
9	0.058	0.067	0.759	-0.009
10	0.093	0.537	0.160	0.162
11	-0.076	0.133	0.027	0.709
12	0.064	0.691	-0.070	0.259
13	-0.177	-0.095	0.680	0.064
14	0.452	0.207	0.439	0.108
15	0.313	0.455	-0.218	0.074
16	0.120	0.224	-0.185	0.585
17	0.043	-0.026	0.157	0.513
18	0.017	0.644	0.289	-0.214

Reliability of the PPOS Scale

To assess reliability, we calculated the Cronbach's alpha value separately for each factor. The values are shown in Table 5.

Total scale reliability was acceptable ($\alpha = 0.724$), factor 1 showed acceptable, factor 3 borderline, and factors 3 and 4 poor reliability,

DISCUSSION

The validation of the Slovenian version of the PPOS questionnaire provides comprehensive insight into the patient-centered attitudes of family physicians in Slovenia. Our results contribute to the growing body of literature examining the cross-cultural validity of this instrument, originally developed by Krupat et al. (2000) (19,25), to assess physician orientation along a continuum from doctor-centered to PCC.

This study aimed to evaluate the psychometric properties of the Slovenian version of the PPOS

Table 4. Contribution of Individual Components (Statements) of the Scale to Explaining the Total Variance

Item	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	3.780	21.002	21.002	3.780	21.002	21.002	2.648	14.711	14.711
2	2.067	11.484	32.485	2.067	11.484	32.485	2.471	13.726	28.438
3	1.432	7.956	40.441	1.432	7.956	40.441	1.762	9.788	38.226
4	1.283	7.130	47.571	1.283	7.130	47.571	1.682	9.345	47.571
5	1.194	6.632	54.203						
6	1.100	6.110	60.313						
7	0.935	5.194	65.507						
8	0.876	4.869	70.376						
9	0.806	4.477	74.853						
10	0.725	4.030	78.883						
11	0.675	3.751	82.634						
12	0.606	3.366	86.000						
13	0.569	3.161	89.161						
14	0.512	2.846	92.007						
15	0.501	2.782	94.790						
16	0.335	1.859	96.648						
17	0.327	1.817	98.465						
18	0.276	1.535	100.000						

Extraction Method: Principal Component Analysis.

Table 5. Cronbach's Alpha Values for the 4-Factor PPOS Scale

Factor	Cronbach's Alpha
Factor 1	0.709
Factor 2	0.688
Factor 3	0.437
Factor 4	0.437
Total	0.724

questionnaire among family physicians, as instrument widely used internationally to assess attitudes toward PCC (24, 27,28). Despite the growing emphasis on PCC in medical theory and policy, our analysis reveals that Slovenian family physicians predominantly exhibit a practitioner-oriented attitude, with a mean total PPOS score of 3.2, indicating a traditional, biomedical approach to care.

Specifically, only two items—Item 9 ("Patients should be treated as if they were partners in the treatment process") and Item 13 ("A treatment plan cannot succeed if it is in conflict with a patient's lifestyle or values")—scored above 5.0, reflecting patient-oriented attitudes that endorse partnership in decision-making and alignment of treatment with patient values (29,30). These findings align with international trends toward holistic and collaborative primary care models (31). However, the majority of items (15 out of 18) remained in the practitioner-oriented range (<4.57), suggesting persistent paternalistic norms in Slovenian family medicine, where the physician assumes a dominant role and patient involvement is limited. This contrasts with Western European counterparts, where PCC is more broadly emphasized (28).

This predominant practitioner orientation was somewhat unexpected given family medicine's traditional association with holistic, continuous, and partnership-based care. Nevertheless, it resonates with broader international patterns where physician attitudes often diverge from the theoretical ideals of PCC. For example, Krupat et al., the original developers of the PPOS, documented a range of scores among US physicians, with many leaning towards a doctor-led model (19,25). Similar findings emerged from cross-cultural studies in Europe (Portugal, Germany, Spain) (33-36), China (37), Middle East and North Africa (38), highlighting systemic, cultural, and hierarchical factors that sustain paternalistic attitudes despite PCC's conceptual endorsement.

Several factors may explain why Slovenian family physicians maintain a practitioner-oriented stance. High patient loads, administrative burdens, short consultation time, limited support in primary care, systemic pressure, ethical dilemmas or unintended consequences (e.g., increased workload for already stretched family physicians) constrain opportunities for shared decision-making and individualized care, leading physicians to default to more directive, paternalistic styles despite conceptual support for patient-centeredness (31,32). This disconnects between medical education which increasingly emphasizes communication and shared decision-making and clinical practice reflects practical limitations and ethical dilemmas related to resource scarcity and professional burnout (31,32). Furthermore, cultural differences and translation challenges may affect how certain PPOS items are interpreted, potentially biasing results toward a more practitioner-oriented view (36).

The predominance of female physicians (69.6%) and high proportion of physicians engaged in teaching (77.7%) in the sample may also influence the findings, as previous research indicates that female and academic physicians tend to score higher on patient-centered measures (39,40). Still, despite these factors, the majority of responses remained in the practitioner-oriented range, suggesting a broader cultural trend that may be resistant to change.

Regarding the psychometric analysis, a Principal Component Analysis (PCA) was applied to the Slovenian PPOS data (n=112) to explore its latent structure. PCA allowed us to identify initial factor patterns and reduce dimensionality, providing an exploratory foundation for further analysis. PCA identified a four-factor model explaining 47.6% of total variance, slightly below the conventional 50% threshold for psychological scale validation (41). While this suggests some underlying dimensions, PCA is limited in its inability to separate shared

and unique variances, thus highlighting the need for future research to adopt more robust methods such as Exploratory Factor Analysis (EFA) and Confirmatory Factor Analysis (CFA) with larger samples (19,42-45). Internal consistency for the total scale was acceptable (Cronbach's α = 0.724), with Factor 1 also demonstrating acceptable reliability (α = 0.709). However, Factors 3 and 4 showed poor reliability (α = 0.437), consistent with prior international findings that PPOS subscale reliability varies across cultures and populations (46,47). This variability suggests some items may lack cultural resonance or clarity, underscoring the importance of ongoing item refinement and rigorous cross-cultural validation, including forward-backward translation and cognitive interviewing (43,44,48-51).

LIMITATIONS AND FUTURE DIRECTIONS

The relatively low response rate (9.5%) poses a limitation in terms of generalizability. Those who responded may already have a stronger interest in PCC, potentially skewing the results positively. Additionally, the current analysis relied solely on EFA. Future studies should employ CFA to test the validity of the emergent four-factor model and assess model fit using indices such as Root Mean Square Error of Approximation (RMSEA), Comparative Fit Index (CFI), and Tucker-Lewis Index (TLI), also known as the Non-Normed Fit Index (NNFI) (46).

Moreover, qualitative research methods (e.g., interviews or focus groups) could complement the quantitative findings by exploring how Slovenian family physicians conceptualize PCC and identify barriers to its implementation. Given the mixed reliability results, a revision of the Slovenian PPOS version may be warranted to improve clarity, cultural relevance, and psychometric performance.

The Slovenian findings thus contribute both local insight and global relevance, illustrating the persistent challenge of translating PCC values into clinical practice amid systemic and cultural constraints. They also emphasize the need for tailored educational and structural interventions to support more patient-centered attitudes and behaviors. Future research should expand sample sizes for robust factor analyses, and refine the PPOS for cultural specificity (52).

Recognizing factors such as systemic pressures and ethical dilemmas or unintended consequences (e.g., increased workload for already stretched professionals) highlights the importance of addressing organizational and systemic reforms alongside individual-level interventions to support meaningful PCC. Future research should incorporate qualitative insights to better understand these complex dynamics and identify strategies that balance workload demands with quality care.

Finally, due to the cross-sectional nature of the study, we cannot assess changes in physician attitudes over time or as a result of professional development. Longitudinal research would be necessary to examine whether targeted education or system reforms can shift attitudes toward more patient-centered approaches.

CONCLUSIONS

Preliminary validation of the Slovenian PPOS reveals acceptable overall reliability and a plausible factor structure but also highlights areas for improvement. While family physicians in Slovenia recognize patient autonomy in principle, a predominantly practitioner-oriented approach remains, influenced by systemic pressures and cultural norms. Refining the PPOS subscales, adapting items culturally, and validating the instrument using advanced psychometric techniques are essential next steps to ensure it accurately reflects Slovenian family physicians' attitudes towards PCC.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest related to the research.

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The research was approved by the Medical Ethics Commission of the Republic of Slovenia (approval number: 0120-429/2022/7).

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Obravnava novorojenčkov s prirojenimi srčnimi napakami v severovzhodnem delu Slovenije: enocentrična retrospektivna raziskava

Management of Newborns with Congenital Heart Defects in Northeast Slovenia: A Single-Center Retrospective Study

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Izvleček

Namen: *Primerjati epidemiološke in klinične lastnosti med bolniki s kritičnimi in nekritičnimi prirojenimi srčnimi napakami (PSN), ob tem pa primerjati še stopnjo prepoznave PSN, čas ukrepanja in izide zdravljenja z obstoječimi podatki iz literature.*

Metode: *Izvedli smo retrospektivno opazovalno raziskavo novorojenčkov s PSN, ki so se rodili v porodnišnici Univerzitetnega kliničnega centra Maribor od leta 2018 do konca leta 2022, kar je skupno 353 bolnikov. Podatki so bili zbrani iz podatkovne zbirke zdravstvene obravnave bolnikov Univerzitetnega kliničnega centra Maribor in iz osebnih kartotek bolnikov. Primerjali smo lastnosti mater in otrok med skupino kritičnih in nekritičnih PSN ter čas prepoznave*

Abstract

Purpuse: *To compare the epidemiological and clinical characteristics between critical and noncritical congenital heart defects (CHD), and the detection rate of CHD, time of intervention, and outcomes in Slovenia and globally.*

Methods: *We performed a retrospective observational study of 353 neonates with CHD who were born in the maternity ward at the University Medical Center Maribor between 2018 and 2022. Data were collected from the database at the University Medical Center Maribor and from personal patient files. We compared maternal and infant characteristics between critical and noncritical CHD. The detection rate of CHD, time of intervention, treatment options, and outcomes were also compared among infants*

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PSN, vrsto zdravljenja in izide zdravljenja glede na podatke iz literature.

Rezultati: *Skupna incidenca kritičnih PSN je bila 1,75/1.000 živorojenih otrok in incidenca pomembnih PSN 4,4/1.000 živorojenih otrok. Genetske nepravilnosti so bile najdene pri 21,4 % (n = 3) otrok s kritičnimi PSN. Kritične PSN so bile prepoznane pred odpustom iz porodnišnice v 85,7 % (n = 12), po odpustu iz porodnišnice pa v 14,3 % (n = 2). Vse kritične PSN so bile prepoznane na podlagi klinične slike in potrjene z ultrazvokom srca, nobena kritična PSN ni bila prepoznana na podlagi presejalnega testa s pulzno oksimetrijo. 27,5 % (n = 97) nosečnic, ki so rodile otroka s PSN, je imelo nosečnostno sladkorno bolezen.*

Zaključek: *Obravnava novorojenčkov s PSN zahteva ustrezno izobražen multidisciplinarni zdravstveni tim, saj zgodnja prepoznava zmanjšuje obolevnost in umrljivost otrok s PSN. Večina PSN je prepoznanih na podlagi klinične slike in potrjenih z ultrazvokom srca. Stopnja prepoznave PSN je zadovoljiva, kljub temu pa še vedno nekaj novorojenčkov s kritičnimi PSN ob odpustu iz porodnišnice ostane neprepoznanih. Delež neprepoznanih kritičnih PSN bi se lahko še povečal, v kolikor se bo nadaljeval trend zgodnjega odpusta novorojenčkov iz porodnišnice.*

with CHD.

Results: *The overall incidence of critical CHD was 1.75/1,000 live births, while the incidence of major CHD was 4.4/1,000 live births. Genetic abnormalities were found in 21.4% (n = 3) of children with critical CHD. Critical CHD was identified prior to the patient being released from the maternity ward in 85.7% (n = 12) and after discharge in 14.3% (n = 2). All critical CHD cases were identified based on clinical condition and confirmed by echocardiography. The absence of a heart defect was detected by newborn pulse oximetry screening. A total of 27.5% (n = 97) of pregnancies were affected by gestational diabetes (GDM).*

Conclusions: *The management of newborns with CHD requires a trained multidisciplinary medical team, as early recognition improves morbidity and prevents mortality. Most CHDs are detected based on clinical conditions, with the diagnosis being confirmed by echocardiography. While the detection of CHD was satisfactory, some newborns with critical CHD were undiagnosed and discharged from the maternity ward. The proportion of undetected infants with CHD may increase in the future if the trend toward earlier discharge continues.*

INTRODUCTION

Congenital heart defects (CHD) are some of the most common congenital anomalies. However, the incidence of CHD depends on what types of congenital anomalies are included in patient analyses and on the level of development of the country in regards to the availability of perinatal diagnostics (1). In newborns, CHDs manifest with signs of heart failure, cyanosis, or only as a heart murmur. However, CHDs can also occur without clinical symptoms (2). The timely diagnosis of CHD is essential, otherwise morbidity and mortality can occur (3). According to published data, the incidence of major (significant) CHD is 6–8 per 1,000 live births, and the incidence of all CHD is up to 75 per 1,000 live births, depending on the availability of ultrasound diagnostics (4).

Complex heart defects with more than one defect represent 10–15% of all CHD cases. CHD accompanied by other congenital anomalies also occurs in the same proportion (5). Approximately a quarter of major defects are critical heart defects that require surgical or cardiological interventional treatment in the first year of life. Risk factors for the occurrence of CHD are chromosomal anomalies and environmental factors, such as gestational diabetes (GDM), rubella virus infection, or reduced folic acid levels (6). An important risk factor is also a positive family history for CHD, such as the presence of CHD in siblings or parents, and especially in the mother (7).

Pulse oximetry screening is a part of the state screening program for newborns in Slovenia, and is performed 24 hours after birth or at least before hospital discharge. Screening consists of measuring arterial blood oxygen saturation using pulse oximetry in two locations, including on the right hand (pre-ductal saturation) and on one leg (post-ductal saturation) (8, 9). The aim of the screening program is the early recognition of significant CHD. Despite all of the screening tests that are performed before or immediately after birth, a large proportion of CHD remains undiagnosed until the development of a critical, life-threatening condition (2). Fetal ultrasound and fetal heart ultrasound monitor heart defects during pregnancy and identify fetuses with CHD that will require delivery in a tertiary center with possible intervention immediately after birth. We must consider the risk of hemodynamic instability, the presence of obstetric complications, and the ability to provide appropriate care in the maternity hospital when we plan the birth of a child with CHD (10).
Neonates with prenatally diagnosed CHD, without expected hemodynamic instability after birth, do not require special preparations; the child is managed in an outpatient clinic. Hemodynamic instability is not expected immediately after birth in the ductal-dependent CHD, but after the closure of ductus arteriosus, which typically occurs 48–72 hours after birth. In ductal-dependent CHD cases, we introduce therapy with prostaglandin E1 (PGE1) and transport the patient to the main tertiary center. The deliveries of newborns with prenatally detected CHD who require immediate intervention after birth should occur in a tertiary center where a neonatologist, a pediatric cardiologist, and a cardiac surgeon are available with the ability for PGE1 administration and the performance of cardiosurgical or cardiological interventional treatment. A newborn with CHD can be delivered vaginally, or by cesarean section; the method of delivery has no effect on the Apgar score or mortality (11, 12). Newborns with CHD who are born close to their due date have fewer complications than those born earlier (12). The objectives of this study were to compare the epidemiological and clinical characteristics between critical and noncritical CHD. We also compared the detection rate of CHD, time of intervention, and outcomes to previous research in Slovenia and globally.

MATERIALS AND METHODS

This retrospective study contained epidemiological and clinical data from neonates with CHD who were born in the maternity ward of the University Medical Center Maribor between 2018 and 2022. We obtained data from the database of the University Medical Center Maribor and from the personal files of patients managed at the University Medical Center Maribor and at the University Medical Center Ljubljana, where cardiosurgical and cardiological interventional treatment of children with CHD occurs. All children underwent echocardiography with precise assessments of cardiac morphology and cardiac function. We classified CHD according to the standard anatomical nomenclature, based on the main morphological features. Major (significant) CHDs were defined as structural abnormalities of the heart or great vessels that have existing or potential functional significance. Among those, CHDs that required intervention in the first year were defined as critical. Premature children with a gestational age of less than 35 weeks who had persistent ductus arteriosus (PDA) or persistent foramen ovale (PFO) as an isolated CHD as part of prematurity without the need for treatment were excluded from the study. The data of 353 patients were reviewed retrospectively. For each patient, we defined the sex, gestational age, birth weight for gestational age, maternal age, presence of GDM, Apgar at 5 minutes, need for respiratory support, need for vasoactive or inotropic support, time of diagnosis, type of CHD, genetic abnormalities, treatment with prostaglandin E1, time of intervention (cardiac catheterization or surgical treatment), and the treatment outcome. Data from the prenatal cardiological assessment and genetic testing were limited in one third of cases. Clear data for fetal echocardiography were available in two cases, and genetic testing was performed prenatally in five cases and postnatally in 14 cases.
Statistical analysis of the data was performed with

IBM SPSS Statistics Standard version 28.0 (IBM Corporation, Armonk, NY, USA) using basic statistical methods, including descriptive statistics, mean values with standard deviations for normally distributed variables, median with confidence interval (95% CI) for non-normally distributed variables, contingency tables, Pearson's chi-square test for descriptive variables and Fisher's correction for small samples, non-parametric Mann-Whitney U test, non-parametric Kruskal-Wallis rank sum test, and post hoc tests with Bonferroni correction. A $p < 0.05$ was considered statistically significant.

RESULTS

During the four-year period, 8,014 children were born in the maternity ward of the University Medical Center Maribor. CHD was identified in 353 cases, of which 51.8% were male and 48.2% were female. The incidence of all CHD was 44/1,000 live births. A total of 24.8% (n = 91) of CHD were significant. When muscular ventricular septal defects (VSD) were excluded, which some studies do, 9.9% (n = 35) of CHD were significant. The incidence of significant CHD was 11/1,000 live births. When cases of muscular VSD were not considered, the incidence of CHD decreased to 4.4/1,000 live births. A total of 4% (n = 14) of cases were critical CHD that needed intervention during the first year of life, 1.7% (n = 6) required intervention during the first month after birth. The overall incidence of critical CHD was 1.75/1,000 live births, while the incidence of critical CHD with intervention in the first month after birth was 0.75/1,000 live births. During the study period, there were 54 stillbirths, of which 59.3% (n = 32) were feticides, and 40.7% (n = 22) were intrauterine fetal demises. In six cases, feticide was performed due to significant CHD (three cases of hypoplastic left heart syndrome (HLHS), one case of univentricular heart, one case of significant aortic stenosis, and one case involving an unspecified complex heart defect). If those cases were added to critical CHD analyses, their incidence would be 2.50/1,000 births. A total of 1.7% (n = 6) of CHD cases were defined as cyanotic and 98.3% (n = 347) were defined as acyanotic. Within the critical CHD cases, 42.9% (n = 6) were cyanotic and 57.1% (n = 8) were acyanotic (Figure 1).

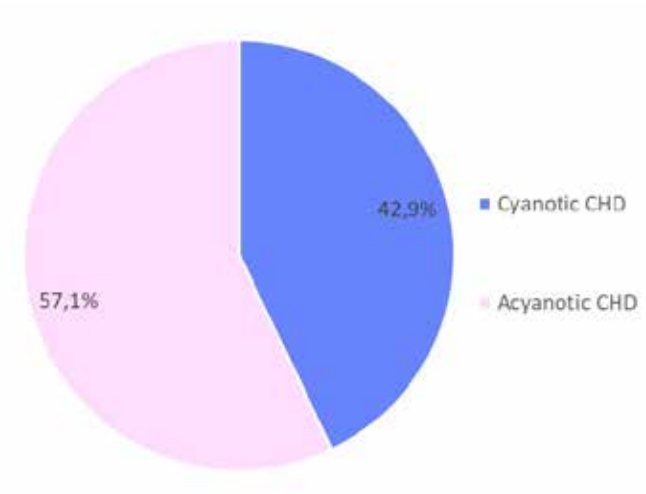

Figure 1. *The percentage of cyanotic and acyanotic congenital heart defects (CHD) within the critical CHD group.*

The distribution of individual acyanotic and cyanotic CHD within the critical and non-critical CHD groups is shown in Table 1.

Therapy with PGE1 for ductal-dependent CHD was necessary in 42.9% (n = 6) of critical CHD cases. The outcome of the treatment of critical CHD was complete repair in 92.9% (n = 13) of patients and staged surgical repair (palliative surgery) in 7.1% (n = 1) of patients. We observed only one death among non-critical CHD patients, which occurred due to complications of extreme prematurity. Genetic abnormalities were identified in 2.5% (n = 9) of children with CHD and in 21.4% (n = 3) of children with critical CHD. The types of genetic abnormalities in individual CHD cases are shown in Table 2.

Critical CHD was identified prior to discharge from the maternity ward or prenatally in 85.7% (n = 12) of cases, and after discharge from the maternity ward in 14.3% (n = 2) of cases (Table 3). All critical CHD cases were identified based on clinical assessments and confirmed by echocardiography. No heart defect was detected by newborn pulse oximetry screening.

Table 1. The distribution of individual acyanotic and cyanotic congenital heart defects (CHD) within the critical and non-critical CHD groups

		Non-critical CHD (N)	Critical CHD (N)
Acyanotic CHD[1]			
Defect type	N (%)	339	8
ASD[2]	133 (37.7)	133	0
VSD[3]	71 (20.1)	68	3
PDA[4]	28 (7.9)	27	1
ASD and PDA	101 (28.6)	101	0
AVSD[5]	0	0	0
PuSt[6]	4 (1.1)	3	1
AoSt7	3 (0.8)	3	0
CoA[8]	1 (0.3)	0	1
Other (MI[9], TI[10], PI[11], CAAs[12])	6 (1.7)	4	2
Cyanotic CHD			
Defect type	N (%)	0	6
ToF[13]	1 (0.3)	0	1
Tricuspid atresia	0	0	0
Pulmonary atresia	0	0	0
Critical PuSt	1 (0.3)	0	1
HLHS[14]	0	0	0
IAA[15]	0	0	0
Critical AoSt	0	0	0
d-TGA[16]	3 (0.8)	0	3
TAPVR[17]	0	0	0
Truncus arteriosus	0	0	0
DORV[18]	1 (0.3)	0	1

[1]CHD – congenital heart defects, [2]ASD – atrial septal defect, [3]VSD – ventricular septal defect, [4]PDA – patent ductus arteriosus, [5]AVSD – atrioventricular septal defect, [6]PuSt – pulmonary stenosis, [7]AoSt – aortic stenosis, [8]CoA – coarctation of the aorta, [9]MI – congenital mitral insufficiency, [10]TI – congenital tricuspid insufficiency, [11]PI – congenital pulmonary insufficiency, [12]CAAs – coronary artery anomalies, [13]ToF – tetralogy of Fallot, [14]HLHS – hypoplastic left heart syndrome, [15]IAA – interrupted aortic arch, [16]d-TGA – transposition of the great arteries, [17]TAPVR – total anomalous pulmonary venous return, [18]DORV – double outlet right ventricle

Table 2. Genetic abnormalities in congenital heart defects (CHD)

Genetic disorder	N	Defect type
Down syndrome	5	DORV[1], ASD[2], VSD[3], ASD and PDA[4]
Noonan syndrome	1	PuSt[5]
DiGeorge syndrome	1	ToF[6]
Sturge-Weber syndrome	1	VSD and PDA
Partial trisomy 16q syndrome	1	Stenosis of pulmonary artery branches

[1]DORV – double outlet right ventricle, [2]ASD – atrial septal defect, [3]VSD – ventricular septal defect, [4]PDA – patent ductus arteriosus, [5]PuSt – pulmonary stenosis, [6]ToF – tetralogy of Fallot

Table 3. Timing of the detection of critical congenital heart defects (CHD)

Time of detection	Critical CHD[1]		
	Acyanotic CHD[1] in N (%)	Cyanotic CHD[1] in N (%)	All in N (%)
Prior to discharge	6 (75)	6 (100)	12 (85.7)
After discharge	2 (25)	0	2 (14.3)

[1]CHD – congenital heart defects

The median time to identify non-critical CHD was 4 days (95% CI: 4–5), while the median time to recognize critical CHD was 3 days (95% CI: 1–6; mode of 1 day; U = 1949.5, p = 0.252). The median time to recognize acyanotic CHD was 4 days (95% CI: 4–5), while the median time to recognize cyanotic CHD was 1 day. The difference in the cyanotic and acyanotic CHD recognition times was statistically different (U = 51.0, p < 0.001).

The median gestational age of non-critical CHD was 37 weeks (95% CI: 37–38), while the median gestational age of critical CHD was 38 weeks (95% CI: 36–40). The difference in gestational age in critical and non-critical CHD was not statistically different (U 2666.0, p = 0.430). The median gestational age of

acyanotic CHD was 37 weeks (95% CI: 37–38), while the median gestational age of cyanotic CHD was 39 weeks (95% CI: 35–39). The difference in gestational age in acyanotic and cyanotic CHD was not statistically different (U 1149.5, p = 0.659).

Apgar at 5 minutes was significantly lower in critical CHD (p < 0.007), in which the Apgar was less than 7 in 21.4% (n = 3) of cases. Neonates with critical CHD needed respiratory support in 50% (n = 7) of cases, including invasive mechanical ventilation in 42.8% (n = 6) of cases, and the addition of oxygen in inhaled air in 7.2% (n = 1) of cases. Vasoactive or inotropic support was necessary in 28.6% (n = 4) of neonates with critical CHD.

In the investigated group of patients from the entire study, 73.9% (n = 261) of newborns were appropriate for gestational age, 15.6% (n = 55) were small for gestational age, and 10.5% (n = 37) were large for gestational age (Figure 2).

A total of 27.5% (n = 97) of pregnancies were affected by GDM in the investigated group of patients from the entire study. There was no statistically significant difference in the incidence of GDM or the adequacy of birth weight for gestational age between critical and non-critical CHD (p = 0.344 and p = 1.000, respectively). No statistically significant difference in the incidence of GDM or in the adequacy of birth weight for gestational age was observed between acyanotic and cyanotic CHD (p = 0.503 and p = 0.482, respectively).

The average age of mothers was 30.9 ± 5.5 years in the investigated group of patients from the entire study. No statistically significant difference in maternal age was observed between critical and non-critical CHD or between acyanotic and cyanotic CHD (U = 2504.5, p = 0.725 and U = 1310.5, p = 0.276, respectively). In the investigated group of patients from the entire study, gestational age was not statistically significantly different relative to the maternal age (r = –0.68, p = 0.200). However, we observed a statistically significant difference in the maternal age according to the history of GDM (H = 7.779, p < 0.020). The average age of mothers was higher in the group of pregnancies affected by GDM, compared to pregnancies without GDM, which was of limited significance as it reflected the established association between advanced maternal age and the higher incidence of GDM.

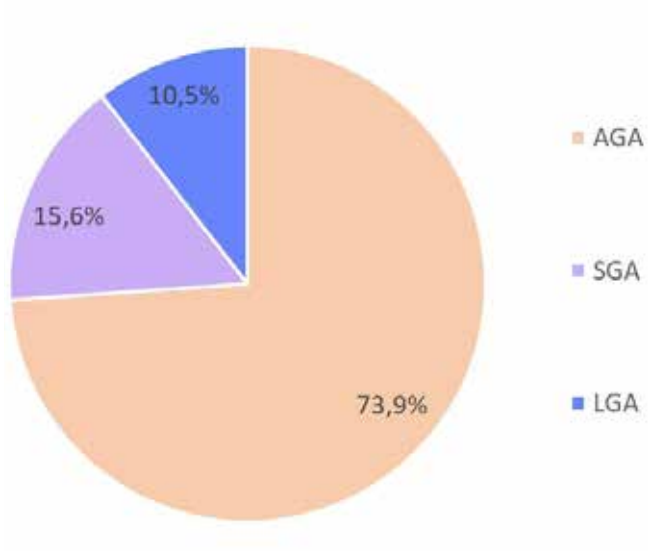

Figure 2. *The percentage of newborns who were appropriate for gestational age (AGA), small for gestational age (SGA), or large for gestational age (LGA) in the investigated group of children with congenital heart defects (CHD) from the entire study.*

DISCUSSION

This study was conducted to evaluate the management of neonates with CHD at the second-largest Slovenian hospital, the University Medical Center Maribor, and to compare treatment outcomes with the existing literature and other studies. The management of newborns with CHD depends on the type of CHD and the time of recognition. We strove to detect significant CHD as soon as possible to plan the childbirth and to provide the best possible obstetric and perinatal care by coordinating work among a multidisciplinary team, including a perinatologist, a neonatologist, and a pediatric cardiologist. Rapid diagnosis and appropriate treatment of newborns with suspected CHD are essential for better outcomes (2, 3).

This retrospective study found a incidence of all CHD in the maternity ward of the University Medical Center Maribor (44/1000 live births) compared to

the existing literature (up to 75/1,000 live births) (4). The incidence of major (significant) CHD (11.4/1,000 live births or 4.4/1,000 live births, if muscular VSDs were excluded) was comparable to data reported in the literature (6–8/1,000 live births) (4, 13). The incidence of critical CHD (1.75/1,000 live births) was slightly lower than that reported in the literature (2.5/1,000 live births) (13, 14), which was most likely due to CHD cases of feticide (30% reduced incidence), and partly due to the management of pregnancies and deliveries with antenatally diagnosed significant CHD at University Medical Centre Ljubljana, where surgical and cardiological interventional treatments are performed.

The proportion of CHD recognized on time (85.7%) was comparable to that reported in a prior Slovenian study (around 90%) (15) and was slightly higher than the European and American average (70–82%) (16-19). We recorded two cases of late recognition, including a combination of coarctation of the aorta and Scimitar syndrome, which manifested at 21 days of age in the form of a blood flow obstruction of the left heart structures. Such an event is also the most common mechanism of unrecognized CHD (20). The second case was a coronary artery anomaly (ALCAPA syndrome), which manifested at two and a half months of age in the form of advanced heart failure. In both cases, the early course after birth was uneventful, and therefore, echocardiography was not performed. All CHD cases were recognized based on the clinical condition, and none were recognized from pulse oximetry screening, which should be further improved and validated.

Genetic abnormalities were observed in 21.4% of critical CHD cases, which was consistent with the literature and other studies (21). Several genetic syndromes are associated with CHD, including Down's syndrome, Noonan syndrome, DiGeorge syndrome, and others, which were also observed in our retrospective study. In the investigated group of children from the entire study, 27.5% of pregnancies were affected by GDM, which was more than that observed in the general population of pregnant women (~10%) (22) and confirmed the correlation between GDM and the increased risk of CHD (23, 24). In developed countries, the prevalence of newborns that are small for gestational age is ~10% (25, 26). In the investigated group of children from the entire study, the percentage of such children was higher at 15.6%, which was also consistent with the other studies (27, 28).

During the study period, screening strategies to detect CHD in Slovenia were based on prenatal fetal ultrasound and physical examinations with echocardiographic confirmation. Pulse oximetry is also performed as an additional screening method for the detection of CHD in all maternity hospitals. The current pre-discharge detection rate of critical CHD is satisfactory at our institution. However, some of the affected neonates still leave the maternity ward undetected.

The main limitations of this study were associated with the centralized management of pregnancies with prenatally known CHD, which affected the incidence of major (significant) and critical CHD outside the capital city. The incidence of CHD was also affected by increasingly developed and accessible prenatal diagnostics and pregnancy terminations. Thus, we must emphasize that in a third of cases, we did not have clear data on prenatal cardiological assessments and genetic testing.

CONCLUSIONS

The management of newborns with CHD requires a trained multidisciplinary medical team, as early recognition improves morbidity and prevents mortality. Most CHD cases are detected based on the clinical condition, and the diagnosis is confirmed by echocardiography. Furthermore, pulse oximetry screening is performed in maternity hospitals nationwide to improve the early detection of critical CHD.

Our study had some limitations, including the small sample size of critical CHD cases. Some of the results were also affected by the redirection of prenatally recognized significant CHD to the main tertiary medical center in Slovenia, the University Medical Center Ljubljana. However, the results of this retrospective study provided insight into the current

state of the treatment of newborns with CHD. Most results were consistent with the existing literature. The detection of CHD was satisfactory, although some newborns with critical CHD were discharged from the maternity ward undiagnosed. The proportion of undetected infants with CHD may increase in the future if the trend toward earlier discharge is continued. There are several possibilities to improve the early detection and management of critical CHD, such as more educated and properly trained medical staff, better ultrasound equipment, standardized screening methods, and efficient cooperation between medical centers. Future research should highlight different types of early medical care for newborns with CHD and record the treatment outcomes nationally. Moreover, the future objective should also be to recognize and expose additional prenatal risk factors for the occurrence of CHD, especially those specific to our environment (pollutants, infections, and maternal substance abuse).

AUTHOR CONTRIBUTIONS:

Conceptualization, T.S.K., T.B. and M.M.; methodology, T.S.K. and M.M.; software, T.S.K. and M.M.; validation, T.S.K. and M.M.; formal analysis, T.S.K; investigation, T.S.K, T.B. and M.M.; resources, T.S.K. and M.M.; data curation, T.S.K. and M.M.; writing the original draft preparation, T.S.K., T.B. and M.M.; writing, review and editing, T.S.K.; visualization, T.S.K.; supervision, M.M. All authors read and approved the final manuscript.

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INFORMED CONSENT STATEMENT:

Not applicable.

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CONFLICTS OF INTEREST:

The authors declare no conflict of interest.

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In vitro primerjava svežih in liofiliziranih 3D natisnjenih materialov za oskrbo ran z vgrajenim ekstraktom *P. major*

Comparative Evaluation of Fresh and Freeze-dried 3D-printed Wound Dressings with *Plantago Major* Extract

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Izvleček

Namen: *Namen študije je bil pripraviti ekstrakt iz zeli širokolistnega trpotca ter oceniti njegovo varnost in vpliv na metabolno aktivnost (MA) fibroblastov v širokem razponu koncentracij. Poleg tega smo preučili učinek suhega in svežega 3D-tiskanega materiala za oskrbo ran (obloge) na fibroblastih, da bi bolje razumeli njegov potencial pri celjenju ran.*

Metode: *Pripravili smo metanolni ekstrakt trpotca in testirali njegov vpliv na MA fibroblastov v širokem razponu koncentracij. S 3D tiskom smo izdelali obloge (sestavljene iz metilceluloze, alginata in nanofibrilirane celuloze) z vgrajenim ekstraktom ter preučili vpliv suhe in sveže obloge na MA fibroblastov in celjenje ran.*

Rezultati: *Ekstrakt je bil varen pri koncentracijah 1–1.000 µg/mL,*

Abstract

Aim: *In this study, we prepared an extract of* Plantago major L. *(PM) herb and assessed its safety and impact on fibroblast metabolic activity (MA) across a broad concentration range. Additionally, we examined the effects of dry and fresh three dimensionally (3D)-printed wound dressings (abbreviated: dressing) on fibroblasts and evaluated their influence on cell migration and proliferation by a scratch assay.*

Methods: *We prepared a methanolic PM extract and investigated its effect on fibroblast MA across various concentrations. Using 3D printing, we fabricated dressings composed of methylcellulose, alginate, and nanofibrillated cellulose, incorporating the extract. Subsequently, we assessed the effects of dry and fresh dressings on fibroblast MA as well as on migration/proliferation by a scratch assay.*

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medtem ko je bil pri 10.000 µg/mL citotoksičen. MA fibroblastov se je povečevala do 1.000 µg/mL, nato je začela padati, kar nakazuje na hormetični učinek. Suha obloga je povečala MA fibroblastov na 1,2 v primerjavi s kontrolo (1) in svežo oblogo (0,99). Test zapiranja raze je pokazal, da obe oblogi podobno učinkovito podpirata migracijo/proliferacijo fibroblastov.

Zaključki: *Naši* in vitro *testi podpirajo tradicionalno uporabo širokolistnega trpotca pri celjenju ran in lahko predstavljajo osnovo za nadaljnje raziskave te rastline v kliničnih študijah.*

Results: *The extract had no cytotoxic effects at concentrations of 1–1,000 µg/mL, but became cytotoxic at 10,000 µg/mL. Fibroblast MA increased up to a concentration of 1,000 µg/mL, after which it began to decline, indicating a hormetic effect. The dry dressing enhanced fibroblast MA 1.2-fold more than the control (normalized to 1.0) and the fresh dressing (0.99-fold). The scratch assay confirmed that both dressings supported fibroblast migration/proliferation equally.*

Conclusions: *Our* in vitro *results support the traditional use of PM in wound healing and can serve as a basis for future investigation of this plant in clinical trials.*

INTRODUCTION

PM has been widely used in traditional medicine for wound healing. Studies have confirmed that it contains bioactive compounds, including plantamajoside, verbascoside, and aucubin, which may contribute to its therapeutic properties (1). However, its cellular effects, including potential hormetic effect (the biphasic response to different concentrations), have not been well documented (2). This study aimed to evaluate the safety and bioactivity of a methanolic extract of PM on fibroblast MA and its potential hormetic effect. Hormesis is described as a biphasic effect, with low dose stimulation and a high dose cytotoxic effect. At low levels, biological, physical, chemical, or physiologic stressors can activate adaptive cellular responses that enhance repair and restoration processes in damaged tissues or organs. These responses frequently result in a performance or recovery level that surpasses that of unstimulated controls—by as much as 30–60% (3). By contrast, higher doses of the same stimuli can become detrimental, leading to cytotoxic effects and impaired function (3).

Additionally, we investigated the integration of PM extract into three-dimensionally (3D)-printed dressings made from methylcellulose (MC), alginate, and nanofibrillated cellulose (NFC). Three-dimensional printing is an emerging technology in wound care that facilitates the fabrication of personalized dressings tailored to the wound's specific shape and size (4). It also enables the precise incorporation of bioactive compounds, making it a promising drug-delivery system (5). The use of multicomponent hydrogels allows for the customization of diverse microstructures and interconnected pore networks, which facilitate efficient oxygen, nutrient, and metabolic waste transport (6). Both natural and synthetic materials can be utilized in 3D printing. Among natural materials, alginate, cellulose derivatives, collagen, and fibrin are widely used because of their biocompatibility, biodegradability, and non-toxicity (6).

The impact of dry and fresh dressings on fibroblast MA and migration was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT) and scratch assays, respectively. Understanding the role of PM extract and its incorporation into advanced biomaterials is essential for developing innovative wound care solutions. By investigating the effects of PM extract on fibroblast function, our study aimed to bridge the gap between traditional medicine and modern wound healing.

MATERIALS AND METHODS

Materials

Dry, crushed herb of PM was purchased from Caelo (LOT 558-1 kg, Germany). Alginic acid sodium salt (ALG, Mw: 80 kDa) and MC (Mw: 658.7 kDa, 25 cP) were obtained from Sigma-Aldrich, Germany. Cellulose nanofibril suspension (NFC, 3% (w/v) at 1.0 g/cm³ aqueous gel, with a nominal fiber width of 50 nm and lengths of several hundred microns) was obtained from The Process Development Center, University of Maine (Orono, USA). Ultra-pure water (Resistivity18.2 mΩ·cm at 25°C) from the ELGA Purelab water purification system (Veolia Water Technologies, UK) was used to prepare all solutions. All other chemicals were of analytical grade.

Methods

Plant material extraction

The dry PM herb was ground into powder with an electric grinder. The plant material was extracted with 70% (v/v) MeOH with a 1:20 plant to solvent extraction medium ratio at 60°C under reflux, with moderate stirring for 2 h. The plant material was removed by vacuum filtration and the filtrate was concentrated under reduced pressure at 40°C (Rotavapor® R-100, BUCHI, Labortechnik AG, Switzerland). The remaining liquid was frozen at –80°C and lyophilized (VirTis BenchTop 6K, SP Industries Inc., PA, USA) overnight to obtain a viscous extract (*extractum spisum*) with a drug extract ratio 5.6:1.

Preparation of 3D printed dressing with added 0.1 wt.% PM viscous extract

A dressing was prepared, consisting of 10 wt.% MC, 5 wt.% ALG), and 1.5 wt.% NFC, a composition shown in our earlier study to be suitable for fibroblast viability and growth (7). For dressing with added extract, 0.1 wt.% PM extract was dissolved in water, which was first mixed with NFC, followed by the addition of ALG and MC. The gel was 3D printed using a pneumatic 3D printer (Vitaprint IRNAS, Slovenia) with extrusion nozzles (Nordson EFD, USA) with a diameter of 0.25 mm. The pressure was set to 4 bars. The dressings were of cylindrical shape with a 20 mm diameter, 1.8 mm height, and layers composed of 18 filaments. The 3D printed dressings were cross-linked with 5 wt.% CaCl2 for 1 min and wiped with a low-lint towel. Some of the dressings were immediately soaked in cell medium (see section: Cell-based testing), while others were frozen at –80°C, and lyophilized overnight (VirTis BenchTop 6K).

Cell-based testing

MTT assay with PM extracts

Human skin fibroblasts (abbreviated as fibroblasts) (ATCC CCL-110™, LGC Standard, United Kingdom) were cultured in Advanced Dulbecco's Modified Eagle's Medium (ADMEM; ThermoFisher, Germany) supplemented with 5% (v/v) fetal bovine serum (FBS), together with penicillin, streptomycin, and glutamine (each at 10% v/v), at 37°C in a humidified atmosphere containing 5% CO2 (7). Fibroblasts were seeded in 96-well microtiter plates at a density of 10,000 cells/well and incubated at 37°C under a 5% CO2 atmosphere for 24 h (8).

The viscous PM extract was prepared as a stock solution of 10 mg/mL (in cell medium), filtered through a 0.22 µm filter, and diluted with the cell medium to obtain final concentrations of 1, 10, 100, 1,000, and 10,000 µg/mL, which were added into respective wells with a final volume of 100 µL. The control well contained fresh medium alone. Cells were incubated for 24 h at 37°C under an atmosphere of 5.0 wt.% CO2.

The MTT assay is one of the most widely applied methods which determines MA as an indicator of cell viability (9, 10). The water-soluble yellow dye MTT is converted to insoluble purple formazan in living cells by mitochondrial reductases. The insoluble formazan is subsequently dissolved in dimethyl sulfoxide (DMSO) and quantified at 570 nm (11). Fibroblasts were used in our study because they are the most abundant dermal cell type and are involved in wound healing. They produce collagen, fibronectin, and proteoglycans, which are major components of the extracellular matrix (12). MTT reagent (10% (v/v) in medium with 5% (v/v) FBS) was added to each

well. After incubation for 3 h and the formation of purple formazan crystals, the medium was carefully discarded, and DMSO (100 µL) was added to dissolve the crystals. Absorbance was determined at 570 nm using a Varioskan Multiple Reader (ThermoFisher Scientific, USA).

All experiments were performed in quadruplicate, with a blank control (sample/control without MTT) for each set. Results are expressed as the mean value with standard deviation (SD). Cell MA was calculated using the following equation:

$$\text{Cell viability} = \frac{(A_s - A_{sb})}{(A_c - A_{cb})} \qquad \textit{Equation 1}$$

where As is the absorbance of the sample, Asb is the absorbance of the sample blank, Ac is the absorbance of the control, and Acb is the blank absorbance of the control.

Dressing biocompatibility by the MTT assay

Cell MA was evaluated according to Mosmann (13), and meeting the ISO 10993-5 and ISO 10993-12 regulations, by the extract method. The assay was performed as described above with the following changes:

Dry dressing containing 0.1 wt.% PM extract and fresh dressing with 0.1 wt.% PM extract were incubated in the cell culture media (ADMEM, supplemented with 5% (v/v) FBS) for 24 h at 37°C under a 5% CO2 atmosphere. The following day, the conditioned medium from the dressing was transferred onto the cells, either undiluted or at a 1:2 dilution, with a final medium volume in each well of 100 µL. Control cells were incubated in fresh cell culture medium supplemented with 5% (v/v) FBS. The remainder of the protocol was unchanged.

Scratch assay with the dressings

The scratch assay is a widely used *in vitro* method for evaluating the wound closure rate by assessing cell migration and proliferation. A scratch is manually created on a confluent cell monolayer using a pipette tip, and cell movement into the wound area is monitored under a microscope (19).

The scratch assay was performed according to (14) and (15). Fibroblasts were seeded in 24-well plates (50,000 cells/well) and incubated overnight at 37°C in ADMEM supplemented with 5% (v/v) FBS under a 5% CO2 atmosphere. Fresh and dry dressings were incubated overnight in 3 mL ADMEM supplemented with 5% (v/v) FBS under identical conditions as in the MTT assay.

The following day, a scratch was created using a 200 µL sterile pipette tip. The cells were washed with fresh medium, which was subsequently discarded. Control wells received fresh medium containing 5% (v/v) FBS, while the medium from the dressing incubation was transferred onto cells in the experimental wells (100 µL). The cells were incubated overnight at 37°C under a 5% CO2 atmosphere.

Images were obtained using an optical microscope (Axiovert 40, Zeiss, Germany) immediately after the scratch was made and at 4 and 24 h post-scratch. All experiments were conducted in triplicate. The area of the wound was quantified using ImageJ software (National Institutes of Health, USA) with the wound healing plugin (16) and the percentage of wound closure was calculated from the following equation:

$$\%\ \text{Wound closure} = \left(\frac{(A_0 - A_n)}{A_0}\right) \times 100 \qquad \textit{Equation 2}$$

where A0 is the initial wound area, and An is the wound area after 4 or 24 h.

Statistical analysis

All numerical values are reported as the mean ± SD. The Shapiro–Wilk test confirmed the normal distribution of experimental data. Levene's test was applied to assess the equality of variances. Because all datasets were well modeled by a normal distribution and homoscedastic, one-way analysis of variance (ANOVA) followed by Dunnetti's post-hoc test was performed accordingly. P-values < 0.05 were considered to be statistically significant. Statistical analysis was performed using SPPS Statistics 27 (IBM Corp., USA).

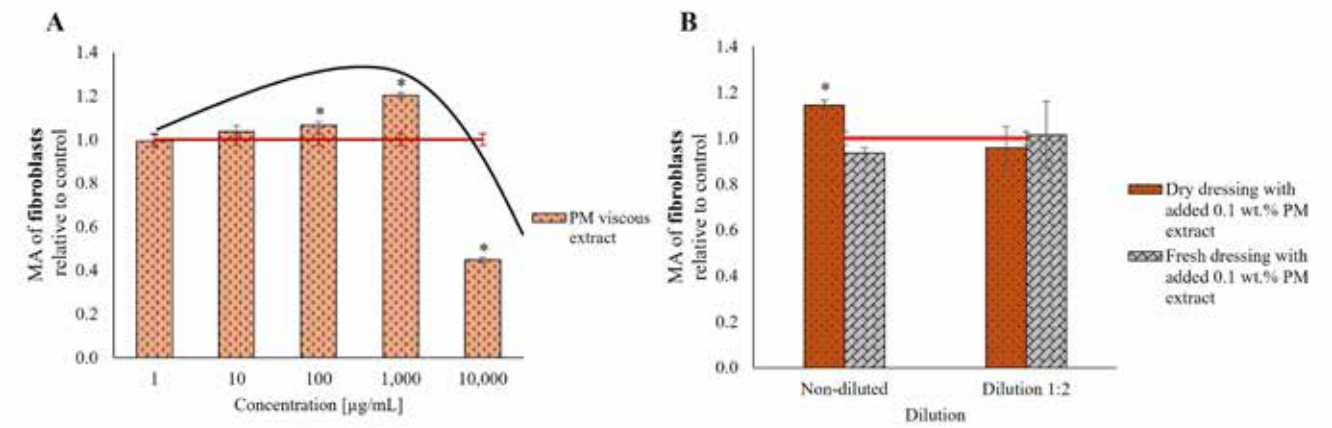

Figure 1. *A: MTT assay with PM viscous extract in a wide range of concentrations. Black curve represents the so-called hormetic effect. B: MTT assay with dry or fresh dressing with added 0.1 wt.% PM viscous extract.-*

RESULTS

MTT assay with PM extract and dressings

The MTT assay results are presented in Figure 1. Part of the results were obtained in the scope of our previous study (17).

Figure 1A illustrates fibroblast MA following treatment with different PM viscous extract concentrations. There was a steady increase in fibroblast MA from 1 to 1,000 µg/mL, followed by major decrease at 10,000 µg/mL, indicating cytotoxicity. This biphasic response suggested the presence of a hormetic effect, characterized by low-dose stimulation and high-dose inhibition. The black curve in the graph represents this hormetic trend, typically showing a 30%–60% greater response compared to the control (18).

Figure 1B illustrates fibroblast MA when treated with conditioned medium from the fresh and dry dressings. The dry dressing increased MA by 1.2-fold compared to the control, indicating enhanced fibroblast activity. However, this effect diminished with 1:2 dilution, reaching MA levels comparable to the control. By contrast, the fresh dressing exhibited a slightly lower relative MA than the control, though this difference was not statistically significant. Upon dilution, MA aligned with that of the control.

Scratch assay with dressing with incorporated PM extract

The migration/proliferation of untreated fibroblasts (control) or in the presence of conditioned medium from the dressings were observed at 0, 4 and 24 h after the scratch was applied. The results are shown in Figure 2. The extent of wound closure was quantified by measuring the scratched area over time. The percentage of wound closure was calculated to compare the effects of different conditions. The results are summarized in Table 1.

Table 1: Percentage wound closure after 4 and 24 h.

	% Wound closure after 4h	% Wound closure after 24h
Control	18.9±13.3	89.4±11.1
Dry dressing with added 0.1 wt.% PM extract	16.9±5.3	86.9±13.2
Fresh dressing with added 0.1 wt.% PM extract	11.5±11.2	85.0±10.0

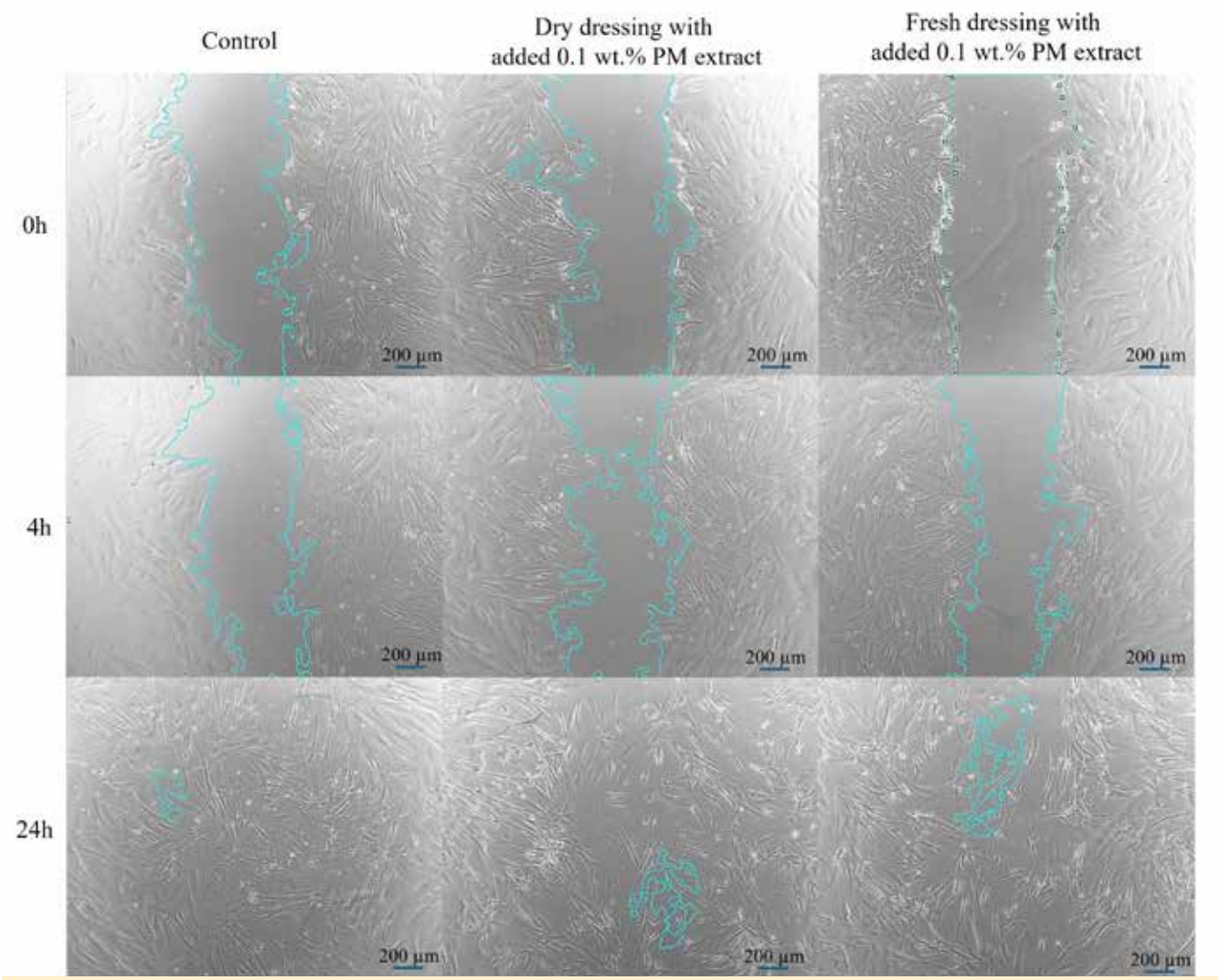

Figure 2. *Scratch assay performed with the conditioned medium from the fresh and dry dressings with added 0.1 wt.% PM extract, compared to control. The micrographs were obtained at 0, 4, and 24 h after scratching, and the percentage wound closure was calculated using ImageJ with the wound healing plugin. The experiments were conducted in three parallel groups. Blue-defined regions represent the area without cells and were used to calculate the wound closure.*

After 24 h, the control wound "healed" with 89.4% ± 11.1% closure, while wounds treated with fresh and dry dressings healed with 86.9% ± 13.2% and 85.0% ± 10.0% closure, respectively. These results indicated no significant difference in wound closure among the groups. While at 4 h the fresh dressing did exhibit a slightly lower average wound closure percentage compared to the control and dry dressing, this difference was not statistically significant. On the basis of the MTT results, the slight reduction in fibroblast MA with the fresh dressing appeared to correlate with slower fibroblast migration in the scratch assay.

DISCUSSION

Hormesis is characterized by a biphasic dose–response, where low concentrations stimulate biological activity,

whereas high concentrations exhibit inhibitory or cytotoxic effects. The observed hormetic effect (Figure 1A) reflected previous findings in various biological systems. Hormesis has been widely reported not only in cellular models, but also in plant growth studies, however, reports specifically addressing this phenomenon in PM remain lacking. For example, Perven et al. demonstrated that an aqueous extract of *Moringa oleifera* promoted shoot growth in *Lepidium sativum* at lower concentrations, whereas higher inhibited shoot growth and reduced root length (20). Hormetic responses to *M. oleifera* have been reported in many other studies, including animal reproduction and sperm preservation (21, 22), neuronal systems (23, 24), bone formation (25), and immune cell responsiveness (26).

Erdal Altıntaş and Aytar Çelik also observed a biphasic effect when testing PM water and ethanol extracts on fibroblast MA (27). Although the authors did not describe it as hormesis, this effect could be clearly observed (Figure 3).

The findings in the present study provided novel evidence of hormetic behavior of PM extracts, which, to our knowledge, has not been previously reported. These insights highlight PM's potential for dose-dependent therapeutic applications, particularly in wound healing, where controlled stimulation of fibroblast activity is beneficial.

The impact of dressing freezing and lyophilization on fibroblast MA was evaluated by testing dry and fresh dressings on fibroblasts cells (Figure 1B). The dry dressing significantly increased MA compared to both the control and the fresh dressing. Upon dilution, the stimulatory effect of the dry dressing diminished. Lyophilization modifies the dressing's structure, enhancing porosity and thus increasing the surface area, which may accelerate and improve the release of bioactive compounds from the PM extract, thereby stimulating fibroblast MA (28). By contrast, the fresh dressing retains more residual moisture, potentially affecting swelling, diffusion, and the gradual release of active compounds into the medium (29). Moreover, in the fresh dressing, stronger interactions between the active PM compounds and the polymer matrix may form, potentially slowing extract release (30).

The scratch assay results demonstrated that the dressings effectively promote wound closure, with almost 90% closure observed after 24 h, comparable to the control (Figure 2, Table 1). This suggested that the dressing provides a favorable environment for cell migration and proliferation, which are crucial for the initial stages of wound healing (31). Zubair et al. conducted a scratch assay using aqueous and ethanolic extracts of fresh and dry PM leaves on oral epithelial cells (32). The authors found that ethanol-based extracts promoted cell proliferation and migration at concentrations of 0.1 and 1.0 mg/mL, but exhibited cytotoxic effects at 10 mg/mL. Notably, the hydroethanolic extract of dry leaves was more effective at 1.0 mg/mL than at 0.1 mg/mL,

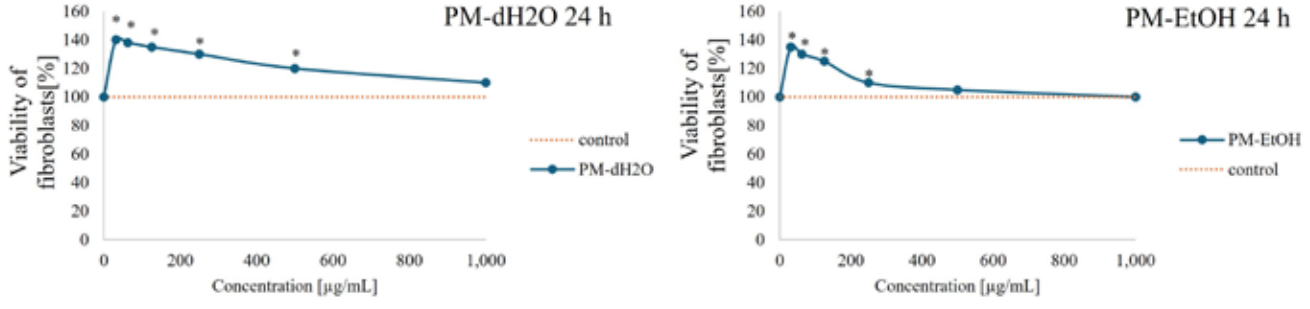

Figure 3. *Hormetic effect of PM water and ethanol extracts. The fibroblast MA was tested with a wide range of concentrations (31.25–1000.0 µg/mL). (The graphs were modified from Erdal Altintas and Aytar Celik with permission. Open access journal, license CC-BY 4.0 (27)).*

achieving 100% wound closure after 24 h compared to 90% at the lower concentration. This suggested a potential hormetic effect of PM, where moderate concentrations enhance cell activity, whereas higher concentrations may be detrimental. The limitation of this study is the use of *in vitro* models and a single cell type, which may not fully represent the complexity of *in vivo* wound healing. Future studies should include *in vivo* validation and broader dose–response analyses to clarify the therapeutic potential and mechanistic basis of PM dressings in wound healing.

CONCLUSION

This study demonstrated that PM extract is safe for fibroblasts at concentrations up to 1,000 µg/mL, with cytotoxic effects observed at 10,000 µg/mL. Notably, to our knowledge, this is the first study to provide evidence of a hormetic effect of PM extracts. The successful incorporation of PM extract into a 3D-printed dressing enabled further evaluation on fibroblasts, revealing that the dry dressing had a more pronounced stimulatory impact on MA compared to the fresh dressing. Dressing lyophilization prolongs its shelf life and enhances the stability of the incorporated extract, allowing the product to be stored at room temperature and making it more suitable for clinical use. Additionally, the scratch assay confirmed that both dressings were equally effective at promoting wound closure, reinforcing the traditional use of PM in wound healing applications. These findings highlight the potential of PM-based biomaterials in advanced wound care and encourage further research into their mechanisms of action and *in vivo* efficacy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

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Utrinki iz predavanj v okviru izvedbe projekta IPANEMA (Integration of PAper-based Nucleic acid testing mEthods into Microfluidic devices for improved biosensing applications)

Highlights from the lectures held as part of the IPANEMA project (Integration of PAper-based Nucleic acid testing mEthods into Microfluidic devices for improved biosensing applications)

Lecture by prof. dr. Ivana Gadjanski, BioSens Institute, Novi Sad, Serbia

Members of the project consortium.

Lecture by prof. dr. Eduardo Corton, University of Buenos Aires, Argentina